

From Department of Microbiology, Tumor and Cell biology,  
Karolinska Institutet, Stockholm, Sweden

**IMMUNE RESPONSES INDUCED BY  
IMMUNIZATION WITH HIV-1 DNA  
FOLLOWED BY HIV-MODIFIED VACCINIA  
VIRUS ANKARA WITH OR WITHOUT  
RECOMBINANT GP140 IN HEALTHY  
TANZANIAN INDIVIDUALS**

Agricola Joachim



**Karolinska  
Institutet**

Stockholm 2017

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet.

Printed by AJ E-Print AB

© Agricola Joachim, 2017

ISBN 978-91-7676-666-8

# **Immune Responses Induced by Immunization with HIV-1 DNA followed by HIV-Modified Vaccinia Virus Ankara with or without Recombinant gp140 in Healthy Tanzanian Individuals**

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

**Agricola Joachim**

*Principal Supervisor:*

Associate Professor Charlotta Nilsson  
Karolinska Institutet  
Department of Laboratory Medicine

*Co-supervisor(s):*

Professor Gunnel Biberfeld  
Karolinska Institutet  
Department of Microbiology, Tumor and Cell  
Biology

Professor Eligius Lyamuya  
Muhimbili University of Health and Allied  
Sciences  
Department of Microbiology and Immunology

Associate Professor Said Aboud  
Muhimbili University of Health and Allied  
Sciences  
Department of Microbiology and Immunology

*Opponent:*

Prof. Dr. med. Hendrik Streeck  
Institute for HIV Research  
University Hospital Essen  
University Duisburg-Essen

*Examination Board:*

Professor Jorma Hinkula  
University of Linköping  
Department of Clinical and Experimental  
Medicine

Professor Francesca Chiodi  
Karolinska Institutet  
Department of Microbiology, Tumor and Cell  
Biology

Professor Patrik Medstrand  
Lund University  
Medical Faculty  
Department of Translational Medicine

**This thesis is dedicated to my family.**

## ABSTRACT

A vaccine against HIV is widely considered the most effective and sustainable way of preventing new infections. We previously conducted a phase I/II clinical trial using multi-clade, multigene HIV-DNA priming and boosting with the recombinant modified vaccinia Ankara (HIV-MVA) virus among healthy Tanzanian volunteers (HIVIS03 trial). The HIV-DNA vaccine contained seven plasmids expressing HIV-1 gp160 subtypes A, B, C, Rev B, Gag A, B and RTmut B and the recombinant HIV-MVA vaccine expressed CRF01\_AE HIV-1 envelope (Env) subtype E and Gag-Pol subtype A. Sixty HIV-uninfected volunteers were randomized into three groups of 20 to receive HIV-DNA or placebo intradermally (id) 1 mg or 3.8 mg intramuscularly (im) at 0, 1 and 3 months with a needle free device and were boosted with HIV-MVA  $10^8$ -pfu or placebo im at 9 and 21 months. The vaccine regimen was safe and induced strong and potent immune responses.

In this thesis we further explored the immune responses elicited by the HIV-DNA and HIV-MVA vaccine regimen and the effect of boosting with a third HIV-MVA or an envelope protein.

In study I, we evaluated the HIV vaccine-induced antibody responses in sera collected from 29 HIVIS03 vaccinees at baseline and four weeks after the second HIV-MVA. High titers of neutralizing antibodies (NAbs) (median 357) were detected using an infectious molecular clone (IMC)/peripheral blood mononuclear cell (PBMC) assay. The NAbs were significantly (but not completely) removed upon depletion of natural killer (NK) cells from PBMC ( $p=0.0039$ ), indicating a role for Fc-receptor mediated antibody function. ADCC-mediating antibodies were demonstrated in the majority (97%) of the vaccinees against CRF01\_AE and/or subtype B. The magnitude of ADCC-mediating antibodies against CM235 CRF01\_AE IMC-infected cells correlated with NAbs against CM235 in the IMC/PBMC assay.

In studies II and III we explored the duration of immune responses in individuals primed with HIV-DNA and boosted with HIV-MVA in the HIVIS03 trial and the effect of a late third HIV-MVA. Twenty volunteers who had previously received three HIV-DNA and two HIV-MVA immunizations in the HIVIS03 trial were given a third HIV-MVA, three years after the second HIV-MVA boost (HIVIS06). A high proportion of vaccinees showed durable binding antibodies, 90% to HIV-1 subtype C gp140 (median titer 200) and 85% to subtype B gp160 (median titer 100) three years after the second HIV-MVA. The majority of vaccinees had detectable ADCC-mediating antibodies, 70% against CRF01\_AE virus-infected cells (median titer 239) and 84% against CRF01\_AE gp120-coated cells (median titer 499). Furthermore, 74% of vaccinees still had IFN- $\gamma$  ELISpot responses, 63% to Gag and 42% to Env. After the third HIV-MVA, there was an increase in Env-binding antibodies and ADCC-mediating antibodies relative to the response seen at the 3-years time point. The frequency of IFN- $\gamma$  ELISpot responses increased to 95% against Gag or Env, and 90% to both Gag and Env,  $p = 0.064$  and  $p = 0.002$ , respectively, after the third HIV-MVA. All 19 (100%) evaluable vaccinees had IgG antibodies to V1V2 CRF01\_AE A244 after the second HIV-MVA with a median titer of 3200. A high proportion (75%) of the vaccinees still had V1V2 IgG antibodies to CRF01\_AE A244 three years after the second HIV-MVA, which increased to 95% after the third HIV-MVA. The magnitude of response before and after the third MVA increased significantly from a median titer of 400 to 1600,  $p<0.0001$ , but not to the same level as after the second HIV-MVA ( $p=0.025$ ). Surface plasmon resonance/Biacore analysis data supported the ELISA findings. V1V2-specific IgG1 antibody responses were more frequently detected than V1V2-specific IgG3 antibodies. Anti-V1V2 IgG1 responses decreased after three years but could be boosted by the third HIV-MVA in the majority of the vaccinees.

In study IV, we evaluated the safety and impact of boosting with subtype C CN54rgp140 Env protein adjuvanted in glucopyranosyl lipid A-aqueous formulation (GLA-AF) in volunteers previously given three HIV-DNA, followed by two HIV-MVA in the TaMoVac 01 trial. Forty volunteers (35 vaccinees and five placebo recipients) were given two CN54rgp140/ GLA-AF immunizations 30–71 weeks after the second HIV-MVA. The vaccine was safe and well tolerated, except for one incident of asymptomatic hypoglycemia. After the second HIV-MVA vaccination, 34 (97%) of the vaccinees developed Env-specific binding antibodies, whereas 79% and 84% exhibited IFN- $\gamma$  ELISpot responses to Gag and Env, respectively. Binding antibodies to subtype C, B and CRF01\_AE Env were significantly boosted by the CN54rgp140/GLA-AF immunizations, while functional antibodies were not significantly boosted. In contrast, T-cell proliferative responses to subtype B MN antigen and IFN- $\gamma$  ELISpot responses to Env peptides were significantly enhanced.

In conclusion, the HIV-DNA prime/HIV-MVA boost regimen elicited potent antibody and cellular immune responses with remarkable durability, and a third HIV-MVA significantly boosted both antibody and cellular immune responses. Binding antibody responses and Env-specific cell-mediated immune responses but not functional antibody responses, increased after boosting with two CN54rgp140/GLA-AF immunizations following priming with HIV-DNA and HIV-MVA.

## LIST OF SCIENTIFIC PAPERS

- I. **Joachim A**, Nilsson C, Aboud S, Bakari M, Lyamuya EF, Robb ML, Marovich MA, Earl P, Moss B, Ochsenbauer C, Wahren B, Mhalu F, Sandström E, Biberfeld G, Ferrari G, Polonis VR. Potent functional antibody responses elicited by HIV-1 DNA priming and boosting with heterologous HIV-1 recombinant MVA in healthy Tanzanian adults. PLoS ONE 2015 10 (4): e0118486.
- II. **Joachim A\***, Munseri P\*, Nilsson C, Bakari M, Aboud S, Lyamuya EF, Tecleab T, Liankia V, Scarlatti G, Robb ML, Earl P, Moss B, Wahren B, Mhalu F, Ferrari G, Sandström E, Biberfeld G. Three-year durability of immune responses induced by HIV-DNA and HIV-modified vaccinia virus Ankara and effect of a late HIV-MVA in Tanzanian volunteers. AIDS Res Hum Retroviruses 2017. Epub ahead of print doi: 10.1089/AID.2016.0251
- III. **Joachim A**, Nilsson C, Msafiri F, Onkar S, Munseri P, Aboud S, Lyamuya EF, Bakari M, Billings E, Robb ML, Wahren B, Mhalu F, Sandström E, Rao M, Biberfeld G. Frequent and durable V1V2 antibody responses induced by HIV-1 DNA priming followed by HIV-MVA boosting in healthy Tanzanian volunteers. Manuscript
- IV. **Joachim A\***, Bauer A\*, Joseph S, Geldmacher C, Munseri P, Aboud S, Missanga M, Mann P, Wahren B, Ferrari G, Polonis V, Robb ML, Weber J, Tatoud R, Maboko L, Hoelscher M, Lyamuya EF, Biberfeld G, Sandström E, Kroidl A, Bakari M, Nilsson C, McCormack S. Boosting with subtype C CN54rgp140 protein adjuvanted with glucopyranosyl lipid adjuvant after priming with HIV-DNA and HIV-MVA is safe and enhances immune responses: a phase I trial. PLoS ONE 2016 11(5): e0155702

# CONTENTS

1	GENERAL BACKGROUND .....	1
1.1	Introduction.....	1
1.2	The epidemiology of HIV.....	1
1.2.1	Global situation .....	1
1.2.2	HIV infection in Sub-Saharan Africa .....	1
1.2.3	HIV and AIDS in Tanzania .....	2
1.3	HIV virology .....	2
1.3.1	Structure and replication of HIV .....	2
1.3.2	HIV diversity and subtypes.....	4
1.4	HIV and AIDS .....	4
1.4.1	Natural history of HIV infection.....	4
1.4.2	Modes of HIV transmission.....	6
1.4.3	Immunopathogenesis of HIV infection .....	7
1.4.4	Laboratory diagnosis of HIV infection.....	7
1.5	Innate immunity.....	8
1.6	Adaptive immunity .....	9
1.7	Immune responses to HIV infection.....	9
1.7.1	HIV-specific humoral immune response.....	9
1.7.2	HIV- specific cellular immune response .....	10
1.8	HIV prevention and vaccine .....	11
1.8.1	Prevention of HIV infection .....	11
1.8.2	HIV vaccine strategies .....	11
1.8.3	Possible correlates of protection against HIV infection.....	12
1.8.4	Lessons learnt from HIV vaccine efficacy trials: Phase IIb and III.....	14
1.8.5	Challenges in developing an HIV vaccine .....	15
2	RATIONALE OF THE STUDY .....	17
3	OBJECTIVES .....	19
4	METHODS .....	20
4.1	Study design and population .....	20
4.2	Vaccines and adjuvant .....	20
4.3	Safety assessment .....	22
4.4	Immunogenicity assessment.....	23
4.4.1	Humoral immunogenicity assessment.....	23
4.4.2	Cellular immunogenicity assessment .....	25
4.5	Data analysis .....	25
4.6	Ethical consideration .....	26
5	RESULTS AND DISCUSSION.....	27
5.1	Functional antibody responses in healthy individuals primed with three HIV-DNA and boosted with two HIV-MVA immunizations (Paper I) .....	27

5.2	Durability of immune responses after three HIV-DNA and two HIV-MVA immunizations and the effect of a late third HIV-MVA immunization (Paper II and III) .....	29
5.3	Safety and immunogenicity of boosting with CN54rgp140/GLA-AF following HIV-DNA/MVA immunizations (Paper IV).....	34
6	CONCLUSIONS, RECOMMENDATIONS AND FUTURE PLANS .....	38
7	ACKNOWLEDGEMENTS .....	40
8	REFERENCES .....	43



## LIST OF ABBREVIATIONS

ADCC	Antibody-dependent cell-mediated cytotoxicity
AIDS	Acquired immunodeficiency syndrome
APC	Antigen presenting cells
ART	Antiretroviral treatment
CMDR	Chiang Mai double recombinant
CRF	Circulating recombinant forms
DC	Dendritic cells
DNA	Deoxyribonucleic Acid
EDCTP	European & Developing Countries Clinical Trial Partnership
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immunospot
Env	Envelope
GLA-AF	Glucopyranosyl lipid A- aqueous formulation
HIV	Human immunodeficiency virus
HIVIS	HIV vaccine immunogenicity study
IFN- $\gamma$	Interferon-gamma
id	Intradermally
IDU	Intravenous drug use
IDUs	Intravenous drug users
IL	Interleukin
im	Intramuscularly
IMC	Infectious molecular clone
LPA	Lymphoproliferation assay
MVA	Modified Vaccinia Virus Ankara
MNH	Muhimbili National Hospital
MUHAS	Muhimbili University of Health and Allied Sciences

MHC	Major histocompatibility complex
MSM	Men who have sex with men
MIP	Macrophage inflammatory proteins
NK	Natural killer
NAb	Neutralizing antibody
NAbs	Neutralizing antibodies
PAMPs	Pathogen associated molecular patterns
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PHA	Phytohaemagglutinin
PRR	Pattern recognition receptors
RT	Reverse transcriptase
RIG-I	Retinoic acid-inducible gene-I
SHIV	Simian/ human immunodeficiency virus
SIV	Simian immunodeficiency virus
SPR	Surface plasmon resonance
TLR	Toll-like receptor
TNF- $\alpha$	Tumor necrosis factor-alpha
WHO	World Health Organization
UNAIDS	United Nations Program on HIV and AIDS
V1V2	Variable region 1 and 2
WRAIR	Walter Reed Army Institute for Research

# **1 GENERAL BACKGROUND**

## **1.1 Introduction**

The acquired immunodeficiency syndrome (AIDS) was first described in the early 1980s in homosexual men in USA [1,2]. The syndrome was defined by unusual diseases, including *Pneumocystis carinii* pneumonia and Kaposi's sarcoma [1,2]. In 1983/1984, the human immunodeficiency virus (HIV) was identified as the cause of AIDS [3,4]. HIV is grouped into two types, HIV-1 and HIV-2 [5,6]. HIV-1 is the major cause of AIDS worldwide. AIDS occurs as results of progressive depletion of CD4<sup>+</sup> T lymphocytes, which are the preferred target of the virus, leading to an immunodeficiency syndrome that paves the way to opportunistic infections and cancers [7,8]. Laboratory diagnosis of HIV infection is mainly based on the detection of HIV antibodies or the HIV antigen and viral nucleic acid. Prevention strategies include behaviour change, safe sex, use of condoms, HIV counselling and testing, screening of blood and blood products, harm reduction efforts for injecting drug users (IDUs), prevention of mother to child transmission by using antiretroviral drugs and male circumcision. The use of antiretroviral treatment (ART) to attack the virus and early treatment of the opportunistic infections help to minimize the risk of transmitting the virus as well as prolong life but does not cure the disease [9]. Development of a safe and effective HIV vaccine would certainly be the best way for the controlling the AIDS pandemic [10].

## **1.2 The epidemiology of HIV infection**

### **1.2.1 Global situation**

HIV continues to be a major global public health concern. In 2015, UNAIDS estimated that 36.7 million (34.0 million–39.8 million) people were living with HIV including 1.8 million children (<15 years old). Since the beginning of the HIV epidemic, approximately 78 million people have become infected with HIV and 35 million people have died from AIDS-related diseases. The number of people worldwide who were newly infected with HIV in 2015 was 2.1 million (1.8 million–2.4 million), a decrease from 3.1 million (3.0 million–3.3 million) in 2000. Globally, new HIV infections have declined by 35% since 2000. In 2015, 1.1 million (0.94–1.3 million) people died from AIDS-related illnesses worldwide compared to 2 million (1.7–2.7 million) in 2005. Access to ART has led to the significant reduction of AIDS-related deaths [11].

### **1.2.2 HIV infection in Sub-Saharan Africa**

The estimated number of people living with HIV in sub-Saharan Africa in 2015 was 25.6 million (range 23.1–28.5 million). Women account for more than half the total number of people living with HIV in sub-Saharan Africa. Sub-Saharan Africa accounts for two-thirds of the global total of new HIV infections. Of the total number of HIV-infected individuals in Sub-Saharan Africa, 19 million (17.7–20.5 million), the vast majority live in East and Southern Africa. This accounts for 46% (960,000 ranging 0.83–1.1 million) of the global

total of new HIV infections in 2015. Between 2010 and 2015, new HIV infections declined by 14% in Eastern and Southern Africa where 470 000 people died of AIDS-related diseases in 2015. The number of AIDS-related deaths in Eastern and Southern Africa fell by 38% between 2010 and 2015 where 54% (10.3 million) people were accessing ART of all people living with HIV in the region [12].

### **1.2.3 HIV and AIDS in Tanzania**

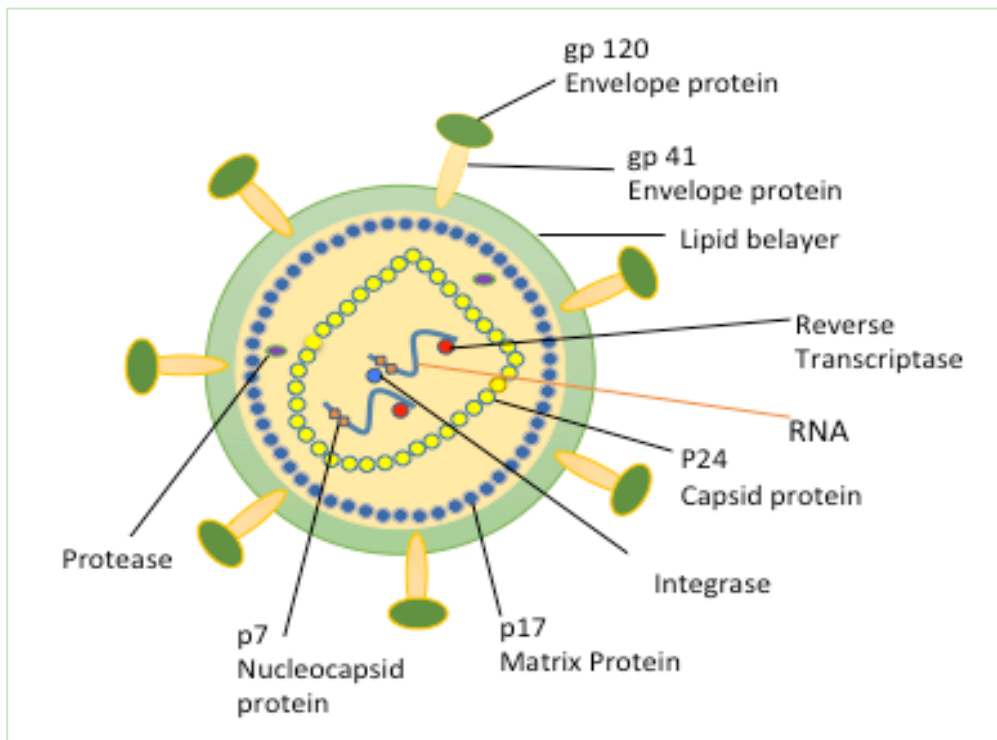
In 2015 an estimated 1.4 million Tanzanians were living with HIV, of whom nearly 28% were children (0-14 years) and 11.2% were young people aged 15-24 years. The overall estimated HIV prevalence is around 4.7% [13,14]. Heterosexual transmissions account for the vast majority (80%) of all HIV infections in Tanzania [15]. The HIV prevalence is higher among women of all age groups compared to their male counterparts (6.2 % among women and 3.8% among men) [16]. These differences are more marked among young women and girls, indicating that women are more vulnerable to HIV infection in Tanzania. In 2015, 54,000 people were newly infected with HIV and 36,000 people died from AIDS-related diseases. Access to ART has helped to minimize the impact of the HIV epidemic. As a consequence, between 2010 and 2015, the number of new infections decreased by more than 20% and the number of people who died from AIDS-related illnesses halved [14].

## **1.3 HIV virology**

### **1.3.1 Structure and replication of HIV**

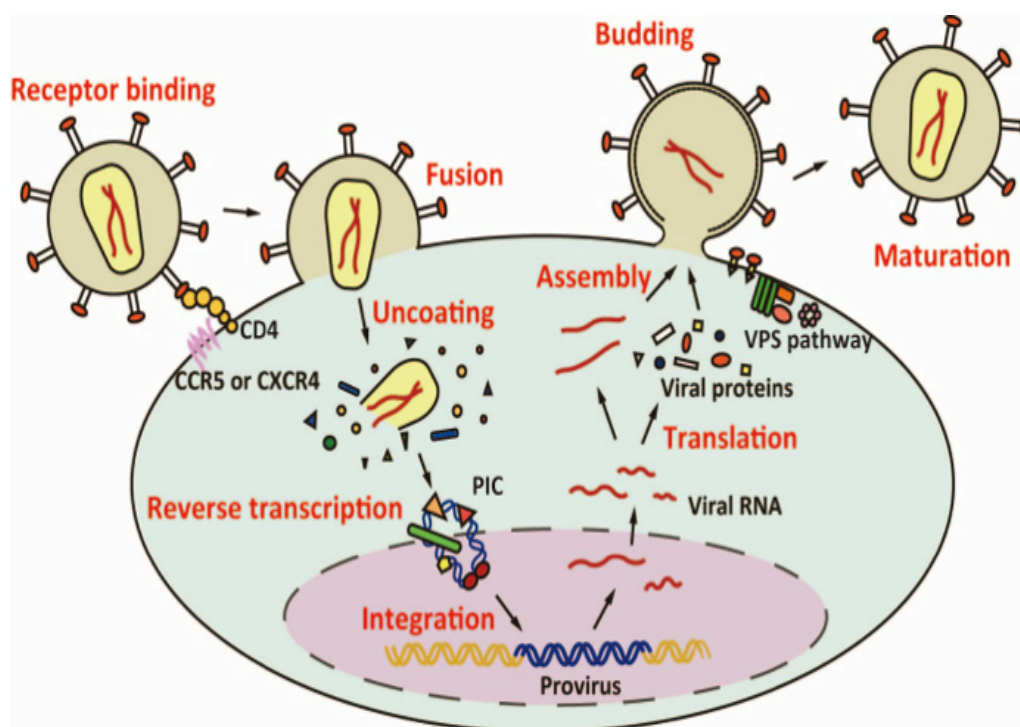
HIV is a member of the Lentivirus genus of the Retroviridae family. HIV-1 was reported to originate from Simian immunodeficiency virus (SIV) cpz from chimpanzees (Pan troglodytes) [17,18] while HIV-2 originated from SIVsmm from sooty mangabeys (Cercopithecus atys) and was transmitted to humans after cross-species infections on multiple occasions [18].

HIV viral particles are spherical with a diameter of 100-120 nm and are surrounded by a lipoprotein rich membrane. Each viral particle membrane includes glycoprotein heterodimer complexes comprised of trimers of the external surface gp120 and the transmembrane gp41 [19,20]. The viral core contains two identical copies of single-stranded positive sense RNA molecules bound to a nucleocapsid (Figure 1). The retrovirus genome consists of the three major structural genes, *gag*, *pol* and *env*. *Gag* encodes the internal structural polyprotein of the virus and it is proteolytically processed into mature proteins; matrix, capsid and nucleocapsid. *Pol* encodes the enzymes reverse transcriptase (RT), integrase that mediates insertion of the provirus into the host genome and protease, which cleaves the precursor Gag polyprotein to produce structural proteins. *Env* encodes the surface glycoprotein and the transmembrane glycoprotein of the virion, which form a complex that interacts specifically with cellular receptor and co-receptor protein. The genome has six regulatory genes including *vif*, *vpr*, *tat*, *rev*, *vpu* and *nef* that encode proteins essential for the viral life cycle [19-21].



**Figure 1. Structure of HIV**

HIV infection commences with the attachment of the virion to the cell surface mediated by an interaction between the extracellular domain of HIV-1 gp120 and cellular receptors [19]. CD4 is the major receptor for HIV-1 and HIV-2 and the transmembrane chemokine receptors CCR5 and CXCR4 are the main HIV-1 co-receptors in vivo. The viral gp120 initially binds to the CD4<sup>+</sup> T cells, which then triggers a conformational change in the host-cell envelope that allows binding of the co-receptor (either CCR5 or CXCR4), which is required for fusion between virus envelope and cell membrane [22]. After binding to the coreceptor, viral and cellular membranes fuse and the viral core is released into the cytoplasm of the cell. After uncoating, the viral RNA is reverse transcribed by the RT enzymes. Subsequently, double-stranded proviral DNA migrates into the cell nucleus and is integrated into the cellular DNA by the integrase. The cellular RNA polymerase transcribes the proviral DNA. The mRNAs are translated by the cellular polysomes. Viral proteins and genomic RNA are transported to the cellular membrane and assemble into immature virions, which are released. Polypeptide precursors are processed by the viral protease to produce mature viral particles [19-22] (Figure 2).



**Figure 2. HIV replication cycle**

<http://www.intechopen.com/source/html/16788/media/image3.jpeg>

### 1.3.2 HIV diversity and subtypes

HIV-1 is classified into three major phylogenetic groups: group M (main), O (outlier), and N (non-M or non-O). Group M, which is responsible for the majority of HIV-1 epidemic infections worldwide is further subdivided into nine HIV-1 subtypes (A to D, F to H and J to K), which are approximately equidistant from one another [23-27]. The most prevalent HIV-1 genetic forms are subtypes A, B, and C, with subtype C accounting for almost 50% of all HIV-1 infections globally. Subtype A viruses are prevalent in areas of central and eastern Africa (Tanzania, Kenya, Uganda and Rwanda) and in Eastern European countries. Subtype B is the main genetic form in western and central Europe, the Americas, Australia, Southeast Asia, northern Africa, and the Middle East. Subtype C is predominant in southern Africa and India, and subtype D in Central Africa [26-30]. The predominant HIV subtypes in Tanzania are A, C and D [31-33]. Up to 55 circulating recombinant forms (CRFs) have been reported around the world with CRF01\_AE, CRF02\_AG and CRF07\_BC being the most common recombinant forms worldwide. Eight HIV-2 (A-H) groups have been identified, group A and B being the most common [7,34].

## 1.4 HIV and AIDS

### 1.4.1 Natural history of HIV infection

The clinical course of HIV infection varies in patients from the time of acute primary infection to AIDS progression. Progression of HIV infection to AIDS leads to progressive reduction in the number of circulating CD4<sup>+</sup> T cells, which over the duration of a number of

years results in immunologic decline and death due to opportunistic infections and neoplasms [35,36] (Figure 3). Progression time from initial HIV-1 infection to AIDS in industrialized countries is approximately 10-11 years in the absence of ART [37,38]. Studies conducted in Africa on the natural history of HIV infection show that the median survival from seroconversion to the development of AIDS is approximately 10 years [39-41]. Similar findings have been reported in the research conducted in developed Western countries [37,38]. However, the study populations differ in terms of age and gender, risk of death before the development of AIDS and access to chemoprophylaxis for opportunistic infections. The mode of transmission also differs among the cohorts, as does the definition of AIDS used by the contrasting studies.

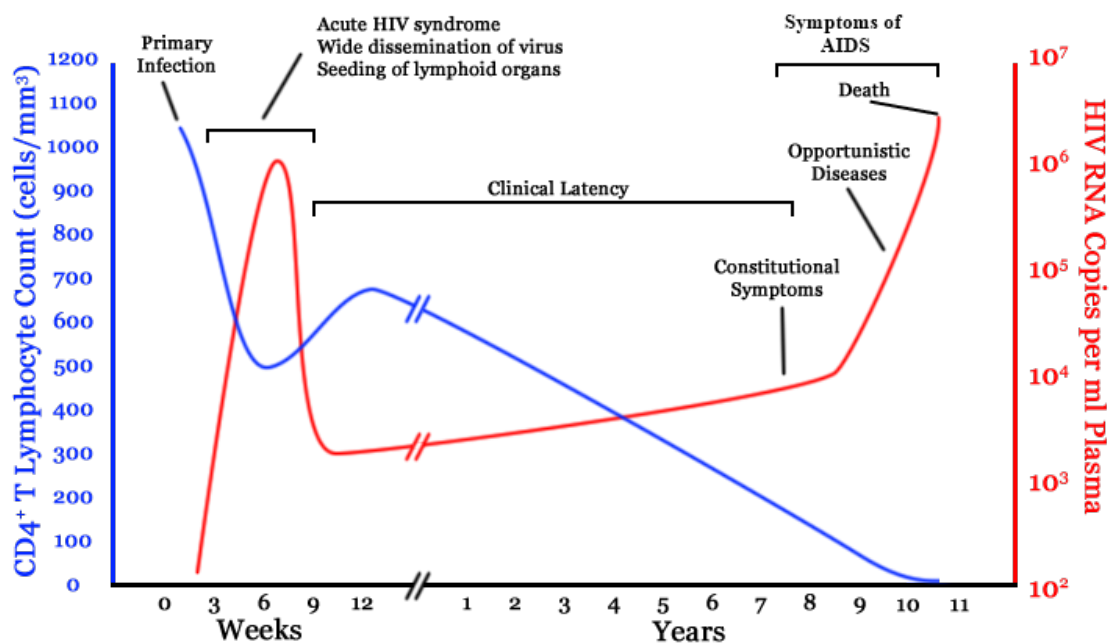
A study by Morgan et al in rural Uganda on individuals infected with HIV-1 showed that the median time from seroconversion to AIDS was 9.4 years and from AIDS to death was 9.2 months. The mode of transmission of HIV infection was mainly heterosexual contact and the median age at seroconversion was 30 years [39]. It was documented in this study that the survival time varied with age at infection where the older patients tended to have shorter survival times compared to younger ones. The reported survival time of patients infected with HIV-1 in studies conducted in Africa is similar to the reported survival of infected patients in developed countries before the use of antiretroviral therapy [39,40].

In healthy HIV-uninfected individuals, the normal CD4<sup>+</sup> T cell count is typically 600–1200 cells/ $\mu$ l. HIV-infected individuals can carry the virus in their blood for several years before developing any serious symptoms. After a certain period of time the viral load increases in the blood, while the number of CD4<sup>+</sup> T cells decline. Use of antiretroviral drugs help to slow down the viral replication and thus preserve CD4<sup>+</sup> T cells and restore the immune system.

In acute primary HIV infection, there are higher levels of the virus in the blood, which spread throughout the body, seeding in various organs, particularly the lymphoid organs. During this phase, HIV may integrate and hide in the host cell's genetic material. In the acute phase, there is a rapid decrease of the CD4<sup>+</sup> T lymphocytes. Most HIV-infected people present with flu-like or mononucleosis-like syndrome approximately 2–4 weeks following HIV infection. Lymphadenopathy occurs in approximately 70% of people with primary HIV infection. These symptoms usually subside within 2–3 weeks whereas generalized lymphadenopathy may persist [42]. During this period, the immune system tries to fight back with killer T cells (CD8<sup>+</sup> T cells) and B-cells producing antibodies. Virus levels in the blood decrease significantly, while levels of CD4<sup>+</sup> T cell tend to rebound as the patient enters the clinically latent stage of disease and for some individuals, the CD4<sup>+</sup> T cell count may rise to the normal level. During clinical latency, HIV-infected individuals may remain free of HIV-related symptoms for several years though the virus continues to replicate in the lymphoid organs. The virus can also remain latent in the macrophages and resting T cells.

The immune system ultimately deteriorates to the point that it is unable to combat other infections. The plasma viral load dramatically rises, while the number of CD4<sup>+</sup> T cells decreases. At this stage, most patients present with symptoms such as severe weight loss and

fatigue. Without ART intervention, the condition progresses to full-blown AIDS, characterized by opportunistic infections, neurological damage, and cancers. Full-blown AIDS occurs when the CD4<sup>+</sup> T cell counts are less than 200 cells/μl of blood. The common cause of morbidity and mortality in HIV-infected patients are mainly opportunistic pulmonary infections including tuberculosis, *Pneumocystis jirovecii* and pneumonia due to *Streptococcus pneumoniae* [43,44]. Other infections like oral candidiasis, cryptococcal meningitis, toxoplasmosis, gastroenteritis and severe viral infection including varicella zoster, cytomegalovirus and herpes simplex infections are also prevalent [43]. AIDS associated malignancies such as Kaposi's sarcoma, Hodgkin's disease, non-Hodgkin's lymphoma and squamous cell carcinomas are frequently reported in chronically immunocompromised patients [45]. The majority of HIV-infected persons die with AIDS and most of them have very low CD4<sup>+</sup> T cell counts at the time of death. Reports of studies conducted in Africa have shown that, HIV-1 subtype D is associated with more rapid disease progression, compared with subtype A, C and G [46-50]. However, a study conducted among Africans and Swedes found no differences in the rate of CD4<sup>+</sup> T cell decline, clinical progression or plasma HIV-1 RNA levels between HIV-infected patients with subtypes A, B, C or D over a mean observation period of 44 months [51].



**Figure 3: Clinical course of HIV-1 infection**

Source: <http://upload.wikimedia.org/wikipedia/commons/a/a4/Hiv-timecourse.png>

### 1.4.2 Modes of HIV transmission

The modes of transmission of HIV are different in different parts of the world and change over time within regions. Heterosexual intercourse is the main mode of HIV transmission in



many resource-poor countries including countries in sub-Saharan Africa [52]. Mother-to-child transmission during pregnancy, at delivery or through breast-feeding, injecting drug use (IDU), sexual contact among men who have sex with men (MSM) and exposure to contaminated blood and blood products account for the remaining infections. In Latin America, most infections are acquired among MSM and through IDU, while heterosexual contact and injection of drugs are the most common modes of HIV transmission in Asia. A high virus load in blood during the acute HIV infection or in the asymptomatic period increases the risk of HIV transmission [53,54].

### **1.4.3 Immunopathogenesis of HIV infection**

The immunodeficiency induced by HIV infection is characterized by the depletion of cells of the immune system; CD4<sup>+</sup> T lymphocytes, macrophages, monocytes and dendritic cells that express CD4. This effect is accompanied by activation of various components of the immune system, resulting in functional immunosuppression (i.e. reduced function of remaining CD4<sup>+</sup> cells) and a form of inflammation that appears to underlie the increased risk of recurrent opportunistic complications occurring in patients with HIV infection. Insufficient immune response to HIV infection fails to control viral replication, provoking continued immune activation [20,55].

During the natural course of the HIV-1 infections, the CD4<sup>+</sup> T cell counts slowly drop, while plasma viremia rises in most patients. Following sexual transmission, HIV infects a mucosal surface and cells of the mucosa-associated lymphoid tissue. The virus binds to the CD4 and the CCR5 chemokine receptors, and infects dendritic cells (DC) and other cells of monocytes-macrophage lineage, as well as peripheral blood T cells. Infection of the CD4<sup>+</sup> T cells, and cell activation by DC, contributes to the spread of HIV-1 within the lymphoid tissues. Reduction in the number of CD4<sup>+</sup> T cells may occur as a consequence of direct HIV-induced cytolysis, T cell-induced immune cytolysis and chronic activation as a result of the HIV antigen challenge leading to rapid terminal differentiation and death of T cells. HIV induces several cytopathic effects that may kill the infected T cells including an accumulation of non-integrated circular DNA copies of the genome, increased permeability of the plasma membrane, syncytia formation and apoptosis. Activated CD4<sup>+</sup> T cells initiate immune response by the release of cytokines required for the activation of macrophages, other T and B cells and natural killer (NK) cells. When CD4<sup>+</sup> T cells are unavailable or not functional antigen-specific cells are incapacitated and humoral responses are uncontrolled. The loss of CD4<sup>+</sup> T cells leads to the occurrence of opportunistic intracellular infections that are the typical characteristics of AIDS [20,35,55,56].

### **1.4.4 Laboratory diagnosis of HIV infection**

HIV infection can be diagnosed by the detection of HIV-specific antibodies or antigen in serum or plasma or detection of viral nucleic acid using polymerase chain reaction (PCR) [57,58]. In Tanzania, the current national HIV testing algorithm includes the use of a simple rapid test (SD-Bioline HIV 1 / 2) for HIV screening followed by a second simple rapid test

(Uni-Gold<sup>TM</sup> HIV) if the first rapid test is positive. HIV enzyme-linked immunosorbent assay (ELISA) is used in case of discordant results between the two rapid tests. PCR is useful in testing infants of HIV-positive mothers because infants may carry maternal anti-HIV antibodies up to 18 months of age [58,59]. HIV RNA PCR is also used for monitoring viral load during ART and to confirm acute HIV infection in a situation where diagnosis has to be made before the usually time for seroconversion [60,61].

## **1.5 Innate immunity**

Innate immunity is the first line of defence against invading pathogens and plays a major role in restricting microbial infection including viruses. Innate immune cells recognize a conformational pattern of an organism rather than a specific epitope. The innate immune system recognizes incoming microbial pathogens through evolutionary conserved pathogen-associated molecular patterns (PAMPs) and responds through intracellular signaling and subsequent cytokines production. The sensing of pathogens especially in viral infection is performed mainly by pattern recognition receptors (PRRs) present either on the cell surface or within distinct intracellular compartments. The PRRs include Toll-like receptors (TLRs), the nucleotides-binding oligomerization domain protein like receptors (NLRs) and retinoic-acid-inducible gene-I (RIG-I) [62,63]. RIG-I-like receptors are cytosolic sensors of viral RNA that respond to viral nucleic acids by inducing the production of the antiviral type 1 interferon. The interaction between PRRs and PAMPs elicits the production of interferon (IFN), and pro-inflammatory cytokines and chemokines that recruit and activate cells of the innate immune system. These include macrophages, NK cells, neutrophils, DC,  $\gamma\delta$  T cells and NK-T cells, which are involved in the inhibition of viral replication in infected cells, as well as to activate adaptive immune response [64]. During HIV infection, the innate immune receptors such as TLR7 and TLR8 are usually activated causing the stimulation of effective DC and the production of IFNs and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) that are involved in the inhibition of viral replication in infected cells [65-67].

Mannose-binding lectins and complement are among the soluble components of the innate immunity, which express anti-HIV activity by directly causing viral lyses and also enhancing viral phagocytosis via macrophages.

NK cells are an important component of the innate immune system that plays a role against HIV infection through its direct cytolytic effector functions against virally infected target cells and non-cytolytic antiviral effector functions. NK cells express soluble factors with antiviral function including IFN- $\gamma$ , perforin, granzymes, FasL and TNF- $\alpha$  [68,69]. In addition NK cells trigger secretion of  $\beta$ -chemokines including MIP1 $\alpha$ , MIP1 $\beta$ , and RANTES, which suppress CCR5-tropic HIV infection by binding to and down-modulating the CCR5 co-receptors [70,71].

## **1.6 Adaptive immunity**

The adaptive immunity system is composed of highly specialized cells that eliminate pathogens. It is specific to a particular pathogen and can also provide long-lasting protection. Adaptive immunity generates immunological memory after an initial encounter with a pathogen and leads to an enhanced response to subsequent encounters with that pathogen. The system includes both humoral immunity and cell-mediated immunity components [72].

Humoral immunity consists of B cells, antibodies, and type 2 helper T cells (Th2). Type 2 helper T cells are included in the humoral immune system because they present antigens to immature B cells, which undergo proliferation to become specific to the presented antigen. B cells are produced and mature in bone marrow tissues, and contain B cell receptors that bind to antigens. B cells produce antibodies that circulate through the plasma. Antibodies bind to pathogens to opsonize them, making it easier for phagocytic cells to bind to and destroy the pathogen. Antibodies neutralize the toxins produced by certain pathogens and provide complement pathway activation, in which circulating proteins are combined in a complex cascade that forms a membrane attack complex on a pathogen cell membrane, which lyses the cell.

Cell-mediated immunity consists of T cells that express either CD4<sup>+</sup> or CD8<sup>+</sup> depending on signals that occur during T cell maturation. Cell-mediated immunity is controlled by type 1 helper T cells (Th1) and cytotoxic T cells. These cells are activated by antigen presenting cells (APC) causing them to mature rapidly into forms specific to the antigen. Helper T cells (Th1) facilitate the organization of immune response by guiding cytotoxic T cells to destroy virus-infected cells. They produce cytokines that guide cytotoxic T cells to pathogens and activate macrophages.

Cytotoxic T cells bind to the major histocompatibility complex (MHC) class I molecules. They destroy the pathogen/virus by releasing granules that contain the cytotoxic perforin and granzyme, which lyse small pores in the membrane of the virus. Helper T cells secrete cytokines such as interferon-gamma, which can activate cytotoxic T cells and macrophages. Memory T cells are created after an adaptive immune response subsides, retaining the presented antigen. They rapidly proliferate and differentiate into helper and cytotoxic T cells that are specific to that antigen should it be detected in the body again [72,73].

## **1.7 Immune responses to HIV infection**

### **1.7.1 HIV-specific humoral immune response**

The first free antibody response to HIV-1 infection is anti-gp41, which appears approximately two weeks after detectable viremia, while anti-gp120 antibodies appear four weeks after detectable plasma viral RNA [74]. These antibodies lack neutralizing capacity and do not appear to have a significant impact on initial control of viremia [75,76]. Antibodies with neutralizing capacity develop later than other immune responses approximately 12 weeks after initial infection and are principally targeted at the envelope

glycoprotein complex, gp41 and gp120 subunits, which mediate viral entry into a CD4<sup>+</sup> T cell. Neutralizing antibodies (NAbs) bind the cell-free virus and prevent infection of host target cells, thus inhibiting viral replication [77,78]. However, because of extensive variability in the envelope gene, mutant viruses rapidly emerge, which are not neutralized by the antibodies that were elicited by the original strain. Broadly NAbs are not routinely made in HIV-1 infection. Studies have shown that during the course of natural infection, both type-specific and broadly cross-reactive NAbs are elicited [79-82]. However, only 15% to 25% of HIV-1-infected individuals will develop broadly NAbs that can mediate neutralization of diverse viral isolates [83-86].

HIV-specific antibodies can eliminate HIV-infected cells via antibody-dependent cellular cytotoxicity (ADCC) in the presence of effector cells. This functional role of antibodies depends on the interaction of Fc-receptors on the surface of effector cells [76,87]. Potential effector cells include NK cells, macrophages, DC,  $\gamma\delta$ T-cells, and neutrophils. ADCC occurs when HIV-1-specific antibodies, bind to their antigens presented on the surface of infected cells. Fc-receptors on NK cells recognize the Fc part of the antibodies. Cytotoxic activities are then induced by the NK cells killing the virus-infected cell [87,88].

### **1.7.2 HIV- specific cellular immune response**

T lymphocytes play an important role in the control of HIV infection. Lack of CD4<sup>+</sup> T cells during HIV infection tends to affect CD8<sup>+</sup> T cell priming, thus reducing viral clearance. After primary infection with HIV, the rapid appearance of cytolytic T cell responses, mainly CD8<sup>+</sup> T cell responses, is associated temporally with a decline in plasma viral load. CD8<sup>+</sup> T cells help to control HIV replication by triggering a cytolytic response. The CD8<sup>+</sup> T cells release perforin and granzymes, which destroy the HIV-infected cells before they produce virions [73,89,90]. CD8<sup>+</sup> T cells can also mediate cytolytic activity against viral targets via expression of Fas ligand by binding to the CD95 on the surface of target cells, which induces apoptotic cell death. CD8<sup>+</sup> T cells also secrete soluble factors with antiviral activity including cytokines (INF- $\gamma$ , TNF- $\alpha$ , IL-2) and beta chemokines (MIP-1 $\alpha$  and MIP-1 $\beta$ ), which bind to CCR5 decreasing the ability of HIV entry into the susceptible cells [91]. An inverse correlation between plasma HIV level and HIV-specific T cell responses has been reported in untreated HIV-infected individuals. Furthermore, long-term non-progressive infection has been associated with both strong HIV-specific cytotoxic T lymphocyte activities and with robust Gag specific CD4<sup>+</sup> T cell proliferative responses [92,93].

The potential role of cytolytic CD4<sup>+</sup> T cells in the control of HIV infection has been reported. The cytolytic CD4<sup>+</sup> T cells express cytolytic markers similar to cytolytic CD8<sup>+</sup> T cells and are distinct from Th1 CD4<sup>+</sup> T cells. Moreover, HIV-specific cytolytic CD4<sup>+</sup> T cells expressing the degranulatory marker (CD107a) have been demonstrated in HIV-infected patients who controlled HIV replication for prolong period of time [94,95].

## 1.8 HIV prevention and vaccine

### 1.8.1 Prevention of HIV infection

Several behavioural and biomedical strategies can reduce HIV-1 infection, and health education plays an important role in tackling the spread of HIV. This includes the provision of education to control transmission, such as the practice of safe sex, monogamous relationships and use of condoms to reduce the risk of exposure to HIV, as well as avoiding needle sharing in intravenous drug abusers and the reuse of needles in clinics. Blood and blood products, tissues and organs must be screened for HIV infection before donation. Other prevention measures include counseling campaigns, screening and treatment of sexually transmitted diseases, male circumcision and the use of ART to prevent mother-to-child transmission [96-98]. Pre-exposure antiretroviral prophylaxis and post-exposure with tenofovir-based regimens has been suggested for HIV prevention in high-risk populations [99,100].

### 1.8.2 HIV vaccine strategies

Several HIV vaccination strategies have been tested in phase I/II trials [101-106]. Live attenuated vaccines are the most potent vaccines against viral infections. The ability of an attenuated viral vaccine to elicit protective immune responses mostly depends on the ability of the virus to replicate in the host. Vaccination with SIV live attenuated vaccines has been used in SIV/ macaque-models resulting in potent immune response that conferred protection [107-110]. However, the use of live attenuated HIV-1 has not been attempted in humans because of safety concerns.

Conventionally, the same vaccines can be administered multiple times as homologous boosts. Vaccines that apply the homologous prime–boost approach include recombinant protein subunit vaccines, (e.g hepatitis B vaccine), polysaccharide vaccines (e.g *Haemophilus influenzae* type b vaccine) and inactivated vaccines (e.g. hepatitis A vaccine). In the search for effective vaccines against HIV infection, new approaches including heterologous prime–boost immunizations have been designed and reported to be more immunogenic than the homologous prime–boost concepts [111,112]. A similar vaccine approach has also been suggested for other diseases such as hepatitis C virus, tuberculosis and malaria. Heterologous prime-boost regimens using DNA and viral vectors or recombinant proteins have been shown to induce broad and potent cellular and humoral responses and to reduce the attenuation effects of viral vector specific immunity [101-104,113].

DNA vaccines have been reported to prime cellular and humoral immune responses, when boosted with recombinant vectors [102-106]. The most common vectors used include modified vaccinia virus Ankara (MVA), fowlpox and adenoviral vectors [114-116]. Various combinations of prime-boosts have been tested in humans including recombinant DNA and the poxvirus vector NYVAC or MVA, adenovirus vectors alone or in combination with plasmid DNA-based vaccines, and were shown to induce strong cellular immune responses

[104,117,118]. In the RV144 efficacy trial, immunizations with canarypox (ALVAC–HIV vCP1521) and HIV envelope protein (AIDSVAX B/E gp120) generated protective immune responses among Thai heterosexual individuals [119].

The use of adjuvants such as IL-12, IL-15, glucopyranosyl lipid A (GLA-AF) and granulocyte macrophage colony-stimulating factor has also been explored to enhance vaccine-induced immune responses [120-122].

Various modes of vaccine delivery have been employed such as either sequential or parallel administration, administration using needle free devices (Bioject or Zetajet), electroporation and/or administration by using a syringe. Vaccines can also be given id, im, intranasally or by the oral route.

### **1.8.3 Possible correlates of protection against HIV infection**

The role of antibodies in preventing HIV-1 acquisition has been suggested by passive and active immunization studies in nonhuman primates [123-125]. Previous studies have shown that passively administered NAb protect against pathogenic chimeric simian/human immunodeficiency virus (SHIV) challenge in rhesus macaque models [123,124]. In addition, HIV-specific monoclonal antibodies infused intravenously to macaques have been shown to protect against intravenous and mucosal chimeric SHIV challenge [126,127]. A study by Haigwood et al found that passively transferred antibodies from HIV-1 infected individuals protected the chimpanzees from HIV-1 infection and rhesus macaques from SIV infection. In addition, early intervention using passive immunoglobulin was reported to control the viral replication and maintain a very low viral load and delay disease progression in SIV infection of macaques [128,129].

Cellular immunity plays a potential role in the control of HIV infection. In a situation where HIV infection occurs after exposure, cellular immune responses will be needed to control virus replication and prevent the development of disease. In HIV and SIV infection a profound expansion of virus specific CD8<sup>+</sup> T cells occurs during acute infection coincident with control of viremia, suggesting a crucial role for CD8<sup>+</sup> T cells in containing viral replication [130,131]. Cytotoxicity, cytokines and phenotypic markers have been reported as potential correlates of CD8<sup>+</sup> T cell immunity. The presence of strong CD4<sup>+</sup> and CD8<sup>+</sup> HIV-1-specific T cell responses in HIV-1-infected individuals that are long-term non-progressors has been reported [132,133].

The importance of CD8<sup>+</sup> T lymphocytes in the control of virus replication has been reported in acute and chronic SIVmac infection of rhesus monkeys [134]. Schmitz et al showed that depletion of CD8<sup>+</sup> T lymphocytes from rhesus monkeys during chronic SIV infection led to marked increase in viremia [130]. In the study where rhesus macaques were challenged with a chimeric SHIV after CD8<sup>+</sup> T cell depletion, a higher viral load and profound CD4<sup>+</sup> T cells depletion were detected in the CD8<sup>+</sup> T cell depleted macaques as compared to monkeys with

intact CD8<sup>+</sup> T cells, further highlighting the importance of CD8<sup>+</sup> T cells in the control of SIV replication [135].

Virus-specific CD8<sup>+</sup> cytotoxic T lymphocyte activity plays an important role in the control of viral replication and subsequently HIV-1 viremia during primary infection, as well as reduction in HIV-1 disease progression [136,137]. HIV-1 specific cytotoxic T lymphocyte responses have been demonstrated in HIV-exposed, persistently seronegative individuals in multiple cohorts [138-141]. Resistance to HIV-1 infection among sex workers who remained HIV-uninfected for many years despite high-risk sexual activity has also been reported in Nairobi, Kenya and elsewhere [142-144]. Goh et al found that the protection against HIV-1 infection in persons with repeated HIV exposure was attributed to T cell immunity rather than inherited CCR5 HIV-1 co-receptor defects [145].

Innate immune responses, such as increased NK cell activity, have been shown to correlate with protection from infection in high-risk cohorts of HIV-exposed but uninfected individuals, including HIV-exposed infants born to HIV-infected mothers, IDUs and HIV-1 discordant couples [146,147]. Increased NK cell degranulation (CD107a) and NK cell activation marker (CD69) expression is also associated with resistance to infection in highly exposed, uninfected individuals [147,148]. Over-expression of inherited protective NK KIR3DL1 and KIR3DS1 receptor alleles in highly exposed seronegative partners of HIV-1-infected subjects has been reported [149].

Mucosal immune responses particularly HIV-1 specific IgA, but not IgG have been demonstrated at mucosal sites as well as in genital tract secretions in exposed HIV-uninfected sex workers and heterosexual partners of HIV-1 infected subjects but not in HIV-1 infected sex workers, suggesting that mucosal antibodies, in the absence of systemic IgG responses, correlate with protection against HIV-1 infection [150].

Higher titers of HIV-1 gp120-specific ADCC-mediating antibodies were reported to correlate inversely with the rate of disease progression. Rapid progressors had significantly lower titers of ADCC-mediating antibodies against HIV-1 gp120 than non-rapid progressors [151]. Likewise, in a cohort of long-term slow-progressors, high ADCC responses were significantly more common than in rapid progressors [152]. Higher levels of ADCC-mediating antibodies were also reported in HIV-1 elite controllers who had undetectable viremia compared to viremic individuals [153,154]. Results from an experimental study, showed that potent ADCC activity developed in rhesus macaques immunized with live-attenuated SIV and was associated with complete protection after pathogenic SIV challenge [107]. Furthermore, studies have shown that the magnitude of ADCC antibody responses correlates inversely with the viral load in acute SIV infection in macaques [155]. In addition, ADCC-mediating antibodies have been shown to protect against HIV-1 infection in mother-to-child transmission [156].

Antigen-specific IgG3 antibodies have been shown to correlate with long-term control of *Plasmodium falciparum* in malaria cases [157]. Early appearance of IgG3 specific NAb in

individuals infected with Chikungunya virus was associated with clearance of the virus and long-term clinical protection [158]. IgG3 has a greater content of neutralizing paratopes compared to other IgG subclasses [159]. Notably IgG1 and IgG3 are known to interact with Fc-mediating receptor functions, including ADCC effector cells, which triggers killing of HIV-infected cells [160]. IgG2 and IgG4 have weak affinities for Fc-receptor function and have been linked with a lack of protection to diseases. Reports suggest that IgG4 responses are associated with progression to AIDS [160].

#### **1.8.4 Lessons learnt from HIV vaccine efficacy trials: Phase IIb and III**

Clues to immune correlates of protection from infection have recently been derived from clinical HIV vaccine phase IIb and III trials.

To date, six HIV vaccine efficacy trials have been conducted worldwide. The VAX004 trial using AIDSVAX® B/B vaccine was conducted among MSM and women at high risk of HIV-1 transmission in the USA and Europe with the aim of inducing humoral immune responses [161]. The AIDSVAX® B/B is a bivalent vaccine containing the monomeric rgp120 HIV-1 envelope antigen from the subtype B MN isolate and gp120 GNE8 in alum. High titers of NAbs elicited against HIV-1 MN tier 1 correlated inversely with risk of HIV infection, but the vaccine did not show protection against HIV-1 acquisition [162]. AIDSVAX® B/E was tested among IDUs in the VAX003 trial conducted in Thailand [163]. The AIDSVAX® B/E vaccine contained rgp120 proteins from subtype B MN strain and CRF01\_AE CM244. The VAX003 vaccine regimen generated high levels of NAbs capable of neutralizing tier 1, but not tier 2 viruses. The vaccine regimen also elicited IgG1 antibodies, very little IgG3 and higher levels of IgG4 [164]. The VAX003 trial did not prevent HIV-1 infection or delay disease progression in IDUs. The Step (HVTN 502) and Phambili (HVTN 503) vaccine trials were designed to test whether the vaccine inducing cell-mediated immune response could prevent HIV infection or reduce plasma viremia following HIV infection. In the Step (HVTN 502) trial, the MRKAd5 gag/pol/nef subtype B vaccine was tested among MSM and high-risk women in North and South America, the Caribbean, and Australia. The vaccine induced IFN- $\gamma$  ELISpot responses in a majority of vaccine recipients, but did not confer protection against HIV acquisition or reduce the plasma viral load after acquisition [165,166]. The same vaccine regimen was also evaluated in the HVTN 503/Phambili study that enrolled heterosexual men and women in South Africa [167]. Similarly, no vaccine efficacy was demonstrated among the vaccine recipients. There was no significant decrease in the HIV-1 plasma viral load in the vaccine recipients compared with the placebo recipients in both the Step and Phambili trials [165-167]. Another vaccine consisting of gag/pol/env subtype A/B/C DNA prime with a matched recombinant Ad5 boost was tested in the HVTN 505 trial among MSM in North America [168]. This vaccine regimen was designed to induce both functional antibodies and cell-mediated immune responses. The vaccine induced polyfunctional CD4<sup>+</sup> and CD8<sup>+</sup> T cells, NAb against easy to neutralize viruses and binding antibodies to multiple subtypes [168,169]. The trial was stopped due to futility and there was no efficacy against HIV-1 acquisition. A study



conducted in non-human primates, using the same vaccine regimen showed a trend of protection against intra-rectal challenge with heterologous SIVsmE660, but not against SIVmac251 infection [170], indicating that the vaccine can protect against a certain virus strain. The RV144 trial, evaluated the protective effect of a prime-boost regimen, consisting of a canarypox-vectored vaccine (ALVAC-HIV vCP1521) and an HIV envelope protein (AIDSVAX® B/E) among HIV-uninfected heterosexual men and women, who were at low risk of acquiring HIV infection in Thailand. The trial showed a modest efficacy of 31.2% at 42 months [119]. This trial gave an insight into the immune correlates of infection risk, which is an important step in the development of an HIV vaccine. The regimen induced binding antibodies against HIV-1 Env, CD4<sup>+</sup> T cell proliferative responses, CD8<sup>+</sup> T cell responses and NAb against T cell line-adapted viruses of subtype B and CRF01\_AE. In the analysis of immune correlates of risk of HIV infection in the RV144 trial, it was found that antibodies against the V1V2 region of HIV-1 Env inversely correlated with the risk of HIV infection, while the presence of IgA Env-binding antibodies was associated with a lack of protection [171]. Moreover, ADCC-mediating antibodies and antibodies to the V3 region correlated with reduced risk of HIV infection in vaccinees with low IgA Env binding antibody titers [171,172]. The IgG1 and IgG3 antibodies targeting the crown of the V2 region of HIV Env were more frequently induced in the RV144 Thai trial compared to the non-protective VAX003 trial [164]. Furthermore, the frequency and magnitude of anti-V1V2 IgG3 responses correlated with decreased risk of HIV-1 infection in the RV144 trial [173]. There was no effect on levels of viral load or CD4<sup>+</sup> T cell count post infection. Notably the vaccination did not affect the clinical course of HIV-1 disease after infection.

### **1.8.5 Challenges in developing an HIV vaccine**

Vaccines are historically the core health intervention for prevention of infectious diseases and would provide the most cost-effective way to reduce HIV-1 infection. However, the development of an effective vaccine against HIV-1 has faced multiple scientific, logistic, economic and social challenges. The ability of HIV to evade host immunity and frequently mutate makes development of an effective vaccine enormously challenging. The extraordinary sequence diversity of HIV-1 makes it difficult to obtain the appropriate immunogens for a vaccine [174,175]. Natural infection does not generate protective immune responses capable to eradicate the virus or prevent progression to disease. The HIV-1 envelope glycoprotein is comprised of variable regions that elicit type-specific NAb with limited breadth, whereas the conserved regions like the CD4 binding site are hidden and poorly exposed to the immune system. Broadly NABs develop in 20% of people within two to three years after HIV infection, but the ability of these antibodies to prevent disease progression is limited [84]. Lack of clear immune correlates of protection in humans is another key challenge, since HIV-1-infected patients are unable to clear the virus. The study assessing the immune-correlates of protection in RV144 generated the hypotheses that V1V2-specific antibodies may have contributed to the protection seen, while high levels of Env-specific IgA antibodies may have weakened the effects of the protective antibodies. There is a possibility that vaccines that are designed to induce higher levels of V1V2 antibodies and

lower levels of Env-specific IgA antibodies may improve the efficacy against HIV-1 infection [171]. Thus the limited although increasing knowledge of immune-correlates of protection in humans call for more research in this area. Lack of predictive animal models limit researchers to study the vaccine's efficacy under various viral challenges, as well as to investigate chemotherapeutic drugs. Chimpanzees have been successfully infected with the virus, and shown to generate immune responses, but they show varied clinical course of infection to humans. SIV and chimeric SHIV infection in rhesus macaques are useful animal models for HIV vaccine studies but so far no appropriate animal models have allowed replication of HIV and disease development similar to that found in humans [176,177]. HIV attacks cells of the immune system preferentially CD4<sup>+</sup> T cells, a specific subset of immune cells that help orchestrate all of the other types of immune responses against pathogens. HIV-1 integrates as a provirus into the chromosomes of long-lived reservoir memory T-cells where it can persist in a latent state narrowing the opportunity for the immune system to clear infection. Infection and destruction of CD4<sup>+</sup> T helper cells weakens the effectiveness of immune responses. There is also a limited interest of the pharmaceutical industry and limited long-term sustained commitment from governments and donors [178].

## 2 RATIONALE OF THE STUDY

Several preventive interventions have been described, including pre-exposure prophylaxis and the use of ART, as prevention for HIV infection but definite control of the HIV epidemic will be achieved mainly with the development of a safe and effective vaccine. Therefore, there is still a need to continue searching for an efficacious vaccine against HIV infection. Several studies using various HIV vaccine strategies have been conducted so far. Heterologous prime boost regimens using DNA, viral vectors, and/or recombinant proteins have been shown to generate HIV-specific cellular and humoral immune responses [101,179,180]. In the RV144 trial that used prime boost strategies, a moderate efficacy of 31.2 % was attained. In the Thai RV144 trial, ADCC–mediating antibodies and antibodies to the V1V2 region of HIV-1 Env correlated with reduced risk of HIV infection in vaccinees with low level of IgA Env binding antibodies. However, the immune responses elicited in the RV144 trial waned quickly [171].

We conducted a phase I/II HIV vaccine trial in Tanzania using priming with multigene, multiclade HIV-DNA and boosting with HIV-MVA among healthy individuals. The vaccines were safe and highly immunogenic. Cellular and humoral immune responses particularly binding antibodies and NAb responses using an IMC/PBMC assay were demonstrated in the majority of vaccinees after three HIV-DNA and two HIV-MVA vaccinations. Study I was conducted to determine the role of NK cells in the NABs detected following the discrepancy shown between IMC/PBMC assay and TZM-bl pseudovirus assay, and explored whether ADCC–mediating antibodies were also elicited by HIV-DNA/MVA vaccine regimen. There is limited data on the longevity of immune responses induced following HIV immunization. Notably, few studies have assessed the duration of immune responses in HIV vaccine trials, as well as whether a late boost has an added value on boosting and maintenance of immune responses, which is needed for long-term protection. Thus, study II and III were conducted to explore the duration of immune responses in a subset of HIVIS03 vaccinees given three HIV-DNA and two HIV-MVA vaccinations. The effect of a late third HIV-MVA was also evaluated in these studies.

DNA vaccines have been shown to prime cellular and humoral immune responses after boosting with recombinant vectors [181]. We have previously reported that intradermal priming with HIV-DNA, followed by boosting with HIV-MVA generated broad and potent immune responses [102]. In the TaMoVac 01 trial, volunteers were primed three times with a standard regimen of five injections of HIV-DNA 1000 µg dose (three Env and two Gag encoding plasmids) versus a simplified regimen of two injections of HIV-DNA 600 µg dose Env and Gag encoding plasmid pools, each delivered separately or combined, and boosted twice with HIV-MVA. The vaccine was safe and cellular immune responses were efficiently primed by the DNA immunizations, irrespective of modality of administration and dose [182]. Study IV was conducted to determine the safety and immunogenicity of adding two adjuvanted Env proteins in volunteers previously given three HIV-DNA/placebo and two

HIV-MVA/placebo vaccinations in the TaMoVac 01 trial. This was the first HIV clinical vaccine trial to combine HIV-DNA, HIV-MVA and adjuvanted Env protein vaccinations.

### **3 OBJECTIVES**

#### **3.1 BROAD OBJECTIVE**

To monitor and characterize humoral and cellular immune responses, induced by an HIV vaccine regimen that combines a plasmid-DNA priming vaccine with a MVA boosting vaccine with or without rgp140 immunization.

#### **3.2 SPECIFIC OBJECTIVES**

- Paper I: To determine the role of NK cells in the neutralizing activity observed in PBMC assays, using sera from the HIVIS03 vaccinated volunteers, and to explore potential Fc –receptor mediated ADCC antibody responses.
- Paper II: To assess the duration of immune responses and the effect of a late third HIV-MVA boost in HIVIS03 volunteers previously given three HIV-DNA and two HIV-MVA immunizations in Dar es Salaam, Tanzania (HIVIS06).
- Paper III: To determine the VIV2 antibody responses induced by HIV-1 DNA priming followed by HIV-MVA boosting in healthy Tanzanian Volunteers (HIVIS03/06).
- Paper IV: To assess safety and the impact of boosting with subtype C rgp140 envelope protein adjuvanted in glucopyranosyl lipid A-aqueous formulation (GLA-AF) in Tanzanian volunteers previously given three HIV-DNA and two HIV-MVA immunizations (TaMoVac 01/rg140).

## 4 METHODS

### 4.1 Study design and population

HIV vaccine-induced immune responses were evaluated in serum or plasma samples, collected from healthy Tanzanian volunteers participating in a phase I/II placebo-controlled double blind trial using multi-clade, multigene HIV-DNA priming and HIV-MVA boosting (HIVIS03/06 trial) (Paper I, II, III). In the HIVIS03 trial, 60 HIV-uninfected volunteers were randomized into three groups of 20 volunteers and received placebo or 1 mg HIV-DNA intradermally (id) or 3.8 mg intramuscularly (im). The DNA plasmids expressing HIV-1 gp160 subtypes A, B, C; Rev B; Gag A, B and RTmut B were given at months 0, 1 and 3 using a needle-free Biojector device. Recombinant MVA expressing CRF01\_AE HIV-1 Env subtype E and Gag-Pol subtype A was delivered im by needle at months 9 and 21. Twenty volunteers, who had previously received three HIV-DNA and two HIV-MVA immunizations in the HIVIS03 trial, were recruited in the HIVIS06 trial and given an additional late third HIV-MVA immunization three years after the second HIV-MVA boost, and followed up for one month (Paper II and III). The vaccination schedule and immunogenicity time points are summarized in Table 1 and Figure 4.

Study IV was built upon the TaMoVac 01 trial, a previous phase IIa randomized placebo double blind clinical trial, conducted in Tanzania [182]. The trial included 120 healthy HIV-uninfected volunteers from two centers, the Muhimbili University of Health and Allied Sciences (MUHAS) in Dar es Salaam, and the National Institute for Medical Research (NIMR)-Mbeya Medical Research Center (NIMR-MMRC) in Mbeya. Participants in the TaMoVac 01 study received HIV-DNA containing seven plasmids expressing HIV-1 gp160 subtypes A, B, and C at weeks 0, 4 and 12 id, using the Zetajet device and were boosted im with HIV-MVA expressing CRF01\_AE at weeks 30 and 46. The plasmids were delivered in combined or separated pools (Pool 1: EnvABC/RevB, Pool 2: GagAB/ RTmutB). In an amendment to the protocol, 40 volunteers (35 vaccinees and five placebo recipients) who received three HIV-DNA/placebo and two HIV-MVA/placebo immunizations were given two CN54rgp140/GLA-AF immunizations im 30-71 weeks after the second HIV-MVA vaccination four weeks apart (TaMoVac/rgp140 trial) (paper IV). Randomization, doses and routes of immunization are summarized in table 2.

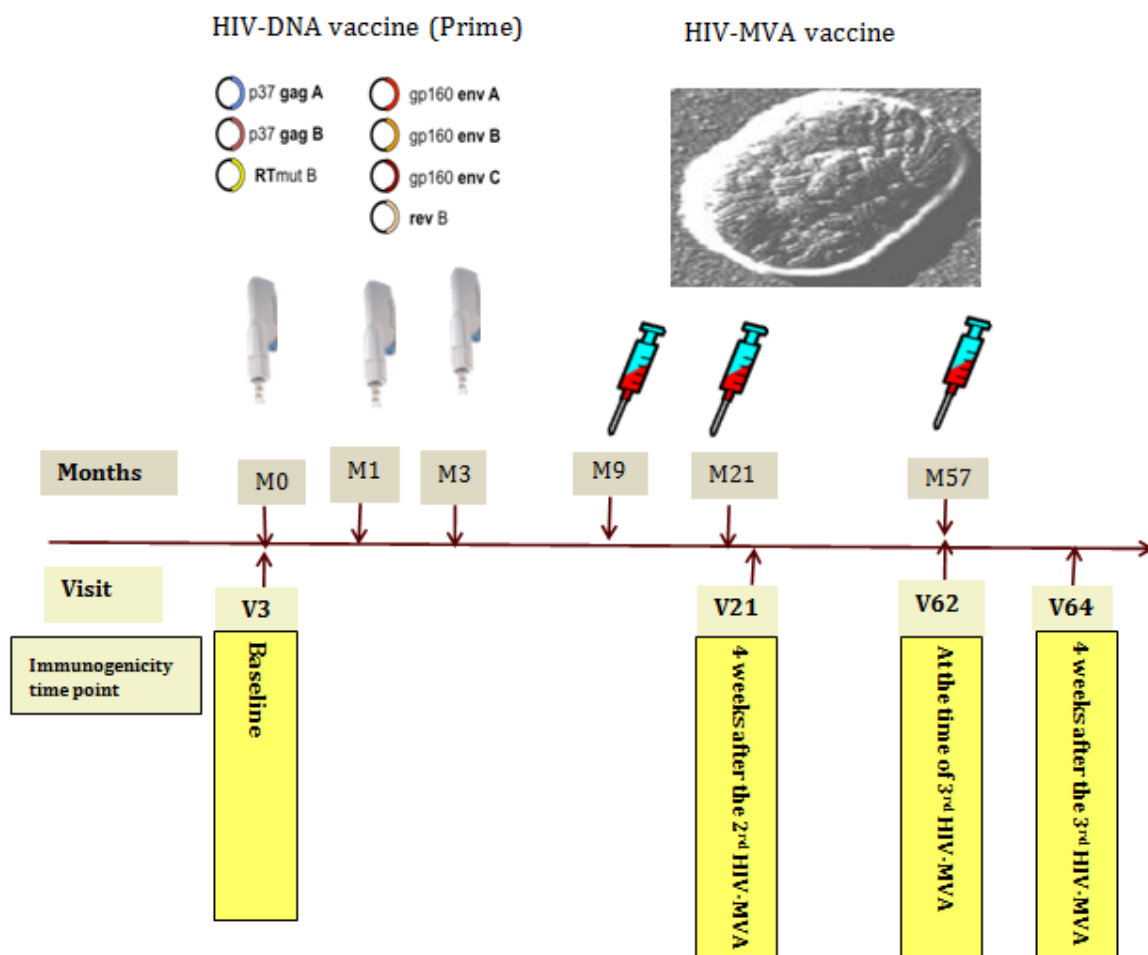
### 4.2 Vaccines and adjuvant

The HIV-1 DNA vaccine was developed by the Wahren research group at the department of virology, Swedish Institute for Infectious Disease Control/Karolinska Institute, Sweden and manufactured by Vecura, (Huddinge, Stockholm, Sweden). The HIV-DNA vaccine was comprised of seven plasmids expressing HIV-1 gp160 subtypes A, B, C; Rev B; Gag A, B and RTmut B (Figure 4) [183,184]. The HIV-MVA vaccine is a recombinant live non-replicating poxvirus vector that was genetically engineered to express HIV-1 gp150 (subtype

E, isolate CM235) and Gag and Pol (integrase-deleted and reverse transcriptase non-functional, subtype A, isolate CM240.) The HIV-MVA was developed by P. Earl and B. Moss from The Laboratory of Viral Diseases, NIAID, NIH USA and manufactured by The Walter Reed Army Institute for Research (WRAIR) Pilot Bio production facility (Forest Glen, MD, USA) [185].

CN54rgp140 is a recombinant subtype C Env protein derived from a Chinese viral isolate 97CM001, clone p97CN54 [186,187]. CN54rgp140 was manufactured according to GMP specifications by Polymun Scientific, Vienna, Austria (accession number AF286226).

GLA-AF is an aqueous formulation containing glucopyranosyl lipid A, a completely synthetic monophosphoryl lipid A (MPL)-like molecule [188]. GLA-AF was manufactured to GMP specifications by The Infectious Disease Research Institute (IDRI) Seattle, USA.



**Figure 4. Vaccines, immunization schedule and immunogenicity follow-up time points (Study 1-III)**

**Table 1. Vaccination schedule for HIVIS03 and HIVIS06 trials**

HIVIS03 trial				HIVIS06 trial	
Vaccine	N	HIV-DNA immunization	HIV-MVA immunization	N	HIV-MVA immunization
Time point		Months 0, 1 and 3	Months 9 and 21		Month 53-59
Group I	20	3.8 mg id	10 <sup>8</sup> pfu im	11	10 <sup>8</sup> pfu im
Group II	20	1 mg id	10 <sup>8</sup> pfu im	9	10 <sup>8</sup> pfu im
Group IIIa	10	Saline im	Saline im		
Group IIIb	10	Saline im	Saline im		

**Table 2. Randomization, doses and routes of immunization (Study IV)**

TaMoVc 01 regimen				Amendment	
Vaccine	N	HIV-DNA ID	HIV-MVA IM	N	Protein IM
Time point		Weeks 0, 4 and 12	Weeks 30 and 46		Two immunizations with 4 weeks interval after week 58
Group I	36	600 µg (combined pools)	10 <sup>8</sup> pfu	10	100 µg CN54gp140 5 µg GLA-AF
Group II	36	600 µg (separate pools)	10 <sup>8</sup> pfu	11	100 µg CN54gp140 5 µg GLA-AF
Group III	36	1000 µg (separate pools)	10 <sup>8</sup> pfu	14	100 µg CN54gp140 5 µg GLA-AF
Group IV	12	Saline ID 2 or 5 x 0.1 ml	Saline IM	5	100 µg CN54gp140 5 µg GLA-AF

N: number of participants. Combined pools refer to a combination of pool 1 (EnvABC/RevB) and pool 2 (GagAB/RTmutB) and separated pools refer to separate administration of pool 1 and 2 into the left and right arm, respectively

### 4.3 Safety assessment

Safety was assessed by solicited local and systemic events, recorded in diary cards following each immunization. Safety assessments were performed at each visit. Information on adverse events recognized to be associated with licensed vaccines was collected on diary cards by participants, for one-week following each vaccination. These included local (pain, redness, swelling and induration) and general events such as fever, headache, malaise, chills, nausea, vomiting, myalgia and arthralgia. Blood samples for laboratory testing were collected at screening, 2-4 and 8 weeks after the final immunization. The laboratory tests included complete blood count, alanine aminotransferase, total and direct bilirubin, random blood glucose and creatinine. Urinalysis, pregnancy and HIV tests were performed at screening, on the day of immunization and on the final visit. Twelve lead electrocardiographs and troponin testing were performed before HIV-MVA vaccination and two weeks post vaccination to monitor for peri-myocarditis.



## 4.4 Immunogenicity assessment

### 4.4.1 Humoral immunogenicity assessment

#### *HIV-specific binding antibodies (Study I, II and IV)*

Testing of binding antibodies to recombinant HIV-1 CM243 CRF01\_AE gp120 (Protein Science Corp), recombinant HIV-1 96ZM651 subtype C gp140 protein (provided by the Centre for AIDS Reagents, NIBSC Potter Bar, UK), HIV-1 IIIB subtype B gp160 (Advanced Biotechnologies, Inc., Columbia, MD) or recombinant CN54gp140 subtype C was performed using an ELISA. Data were reported as reciprocal endpoint titers.

#### *Binding antibodies to scaffolded HIV-1 envelope V1V2 (Study III)*

IgG binding antibodies to scaffolded gp70 V1V2 protein was performed by ELISA, as described in paper III. The testing included recombinant gp70 (MLV)-V1V2 protein (CRF01\_AE A244, subtype C CN54 or subtype B Case A, Immune Technology Corp., New York, USA). Data were expressed as end-point titers, which were calculated as the reciprocal of the highest dilution that yielded an absorbance value above two times the mean of the pre-immunization value.

#### *Assessment of V1V2 specific IgG subclasses (Paper III)*

Testing for IgG specific subclasses of antibodies to gp70 V1V2 CRF01\_AE A244 and gp70 V1V2 Consensus C was performed using ELISA. Results are expressed as end-point titers as described in paper III.

#### *Surface Plasmon resonance (SPR)/Biacore assay (Study III)*

Measurements of antibodies to Cyclic V2 of HIV-1 Env were performed with a Biacore T100 as described in paper III.

#### *Infectious molecular clone (IMC)/PBMC NAb assay (Study I and IV)*

Neutralizing activities were measured using a PBMC assay, employing infectious molecular clones (IMC) carrying the luciferase gene from Renilla reniformis (LucR) as a reporter. The IMCs used were: CM235 CRF01\_AE, SF162 subtype B, BaL subtype B and GS015 subtype C. The method is detailed in study I and IV. The percent neutralization of the post-vaccination serum was calculated based on the level of virus growth in the presence of the same dilution of pre-vaccination serum and neutralization values greater than 50% were considered positive.

#### *A standard PBMC-based NAb assay using a p24 read out (Study II)*

NAb was measured against SF162 subtype B and CM244 CRF01\_AE in a standard PBMC-based NAb assay using a p24 read out as described in study II. Neutralization titers were

defined as the reciprocal of the highest serum dilution, giving at least a 90% reduction of HIV-1 p24-antigen compared to virus control.

#### *TZM-bl pseudovirus NAb assay (Study I, II and IV)*

NAb activities were measured using pseudoviruses and a luciferase-based assay in TZM-bl cells against CM235 CRF01\_AE, SF162 subtype B, and GS015 subtype C (study I & IV) and SF162 and subtype C 93MW965.26 pseudotyped viruses (study II). The assay measures the reduction in luciferase reporter gene expression in TZM-bl cells with a single round of pseudovirus infection. The inhibition percentage was calculated as compared to relative luminescence units (RLU) in the virus control wells, after subtraction of background (cells alone) RLU. A result  $\geq 50\%$  is considered to be a positive response.

#### *IgG depletion from serum (Study I)*

IgG depletion was performed on whole vaccinees sera using protein G Sepharose beads (GE Health care Bio-Science Corp, USA) according to the manufacturer's instructions.

#### *NK cell depletion from PBMC (Study I)*

NK cell depletion was performed on cryopreserved PBMC using mouse anti-human CD16 and CD56 antibodies (Invitrogen, Carlsbad CA) and Dynabeads (M-280) coated with sheep anti-mouse IgG (Invitrogen, Carlsbad CA), as per the manufacturer's instructions. The neutralizing activities were then evaluated using NK cell-depleted PBMC versus bulk PBMC.

#### *Infection of CEM.NKRCCR5 cell line with HIV-1 IMC (Study I, II and IV)*

For ADCC assays, IMCs were titrated in order to achieve maximum expression within 36–48 hours after infection, as described in study I. Assays performed using the infected target cells were considered reliable if the percentage of viable p24+ target cells was  $\geq 20\%$  on the day of testing.

#### *ADCC-GranToxiLux (ADCC-GTL) assay (Study I and II)*

ADCC-mediating antibodies were measured by a flow cytometry GTL-based assay using gp120 coated target cells as described in study I. The CEM.NKRCCR5 target cells were coated with recombinant gp120 HIV-1 protein derived from Env of HIV-1 CM243 CRF01\_AE (GenBank accession no. AY214109; Protein Sciences Corporation) or HIV-1 gp120 SF162 subtype B (GenBank accession no. AAT67508; Immune Technology Corp). PBMCs were obtained from an HIV- seronegative healthy donor. The results were expressed as a percentage of Granzyme B (GzB) activity positive cells. The final results were expressed after subtracting the background from the percentage of GzB activity observed under the conditions containing effector and target cell populations in the presence of vaccinee serum. The ADCC-mediating antibody titer was defined as the reciprocal of the highest dilution indicating a positive GzB response ( $>8\%$  GzB activity) after background subtraction as described by Pollara et al [189].

#### *ADCC-luciferase assay (Study I, II and IV)*

In this assay, measurement of ADCC-mediating antibodies was performed using Env.IMC.LucR virus-infected targets cells as described in study I. The Env-IMC-LucR viruses used were subtype CRF01\_AE HIV-CM235- 2-LucR.T2A.ecto/293T (CM235 IMC) (GenBank accession number AF259954.1) and SF162.LucR.T2A.ecto/293T (SF162 IMC) (GenBank accession number EU123924), respectively. ADCC activity was measured as the percent of loss of luciferase activity observed in the presence of serum. The ADCC-mediating antibody titer was defined as the reciprocal of the highest dilution indicating a positive specific killing (>15% specific killing activity determined based on the responses observed before immunization to allow for 2% false positive rate), after background subtraction.

#### **4.4.2 Cellular immunogenicity assessment**

##### *IFN- $\gamma$ ELISpot assay (Study II and IV)*

IFN- $\gamma$  ELISpot was performed on freshly isolated PBMCs using the h-IFN-gamma ELISpot PLUS kit in a two-step detection system (Mabtech, Nacka, Sweden). Results were expressed as spot forming cells (SFC) per million PBMC. ELISpot responses were considered positive if the number of spot-forming cells was >4 times the background and baseline value and >55 SFC/106 PBMCs. Data were excluded from analyses if the background responses in medium wells exceeded 60 per million PBMCs.

##### *Lymphoproliferation assay (Study IV)*

Tritiated [ $^3\text{H}$ ]-thymidine lymphoproliferation assay (LPA) was performed as described in study IV. T cell proliferation was reported as a stimulation index (SI), determined by dividing the mean counts per minute of the antigen-stimulated wells by the mean of the unstimulated control wells. A SI >6 was considered positive, based on the mean reactivity of 57 healthy Tanzanian volunteers.

#### **4.5 Data analysis**

The safety analysis dataset included all solicited, non-solicited and routine laboratory data that were collected after the first vaccination, up to four to eight weeks after the last vaccination (HIV-MVA or CN54rgp140/ GLA-AF). The solicited and non-solicited events were summarized according to the maximum grade of severity reported. Laboratory events were included if they were new or had increased in grade and summarized by grade.

The analysis of immunological data was performed using GraphPad PRISM version 6 (GraphPad Software, Inc., La Jolla, CA, USA). The Wilcoxon rank test was used to determine statistical significance between bulk and NK cell depleted PBMCs. The Mann-Whitney test was used to compare the magnitudes of ADCC responses, neutralizing titers, binding antibody titers and IFN- $\gamma$  ELISpot responses between the vaccination groups (Study I & IV). For pairwise analysis, the Wilcoxon matched-pair signed rank test was used to

compare the magnitudes of humoral and cellular immune responses before and after the vaccinations (Study II & III). Fischer's exact test was used for comparisons of frequencies. Correlations were determined by the Spearman rank correlation method used for non-parametric data. A two-sided p-value of 0.05 was considered statistically significant.

#### **4.6 Ethical consideration**

The HIVIS03, HIVIS06 and TaMoVac/rgp140 trial protocols were approved by Tanzania's National Health Research Ethics Committee, the Senate Research and Publications Committee of the Muhimbili University of Health and Allied Sciences (MUHAS), and the Mbeya Medical Research Ethics Committee. The Regional Ethics Committee, Stockholm, Sweden, also approved the studies. The Tanzania Food and Drugs Authority approved the use of the vaccine candidate products for humans in Tanzania. All trials were conducted in accordance with the International Council of Harmonization and Good Clinical Practice guidelines. Written informed consent was obtained from all volunteers before enrollment.

## 5 RESULTS AND DISCUSSION

### 5.1 Functional antibody responses in healthy individuals primed with three HIV-DNA and boosted with two HIV-MVA immunizations (Paper I)

We previously conducted a phase I/II HIV vaccine trial (HIVIS03) among healthy adult volunteers in Dar es Salaam, Tanzania, which included priming three times with HIV-DNA followed by boosting twice with HIV-MVA [102]. The majority, 97%, of the vaccinees, exhibited IFN- $\gamma$  ELISpot responses, 93% to Gag and 79% to Env two to four weeks after the second HIV-MVA boost. All (100%) vaccinees had HIV-specific lymphoproliferative responses. Furthermore, 90% had binding antibodies against subtype B gp160 after the second HIV-MVA. There were no NAb demonstrated in the TZM-bl pseudovirus neutralization assay. However, using an IMC/PBMC assay, high NAb response rates were demonstrated, 83% against CM235 CRF01\_AE, 72% against SF162 subtype B and 31% against BaL subtype B.

In paper I, we evaluated the functional antibody responses (NAbs and ADCC-mediating antibodies) in HIVIS03 vaccinees' sera collected at baseline and four weeks after the second HIV-MVA vaccination. We measured the magnitude of NAbs against the CM235 CRF01\_AE. High NAb titers were detected after the second HIV-MVA vaccination (median 357, range 20-2868) using the IMC/PBMC assay, while no NAbs were demonstrated using the pseudovirus/TZM-bl assay. HIV NAbs detected in the IMC/PBMC assay against the CM235 IMC were IgG-mediated. Discrepancies in the NAb results using the two assay platforms have been reported previously [190]. In addition, in a study by Choudhry et al using a monoclonal antibody, cross-clade neutralization was demonstrated in a PBMC assay, but no neutralization in a TZM-bl pseudovirus assay [191]. The possible explanation for the discrepancy is that the TZM-bl pseudovirus assay is a single round assay and mainly measures the inhibition of virus binding and entry compared to an IMC/PBMC assay, which encompasses multiple rounds of infection, thus catching all the potential inhibition stages of the virus replication.

The interaction between antibodies and NK cells has been shown to enhance neutralization of HIV-1 [192,193]. We assessed the potential role of NK cells in the PBMC NAb assay activity. Sera from nine HIVIS03 vaccinees with NAb titer above 200 were tested against the CM235 CRF01\_AE and BaL subtype B IMC using bulk and NK cell-depleted PBMC in the neutralization assay four weeks after the second HIV-MVA boost. The neutralizing activity was significantly removed (but not completely) when using NK cell-depleted PBMC compared to bulk cells ( $p = 0.0039$ ), (figure 2 in paper 1) suggesting a role for Fc-receptor-mediated antibody function. A similar result was also demonstrated when tested against BaL subtype B IMC. The remaining small neutralizing activity detected following NK-cell depletion from PBMC may be attributed to other Fc-receptor-bearing cells in the bulk PBMC, such as monocytes and/or macrophages.

Following the demonstration of the role of NK cells in the neutralizing activity of the vaccinees sera we tested whether ADCC-mediating antibodies were induced by the HIVIS03 vaccine regimen. ADCC activity was measured in sera from 29 vaccinees collected at baseline, two months after the first HIV-MVA and four weeks after the second HIV-MVA boost using both gp120-coated target cells (ADCC-GTL assay) and IMC-infected target cells (ADCC-Luciferase assay), against the vaccine subtype homologous CRF01\_AE (Env E) and subtype B viruses. The results are summarized in Table 3.

**Table 3. Frequency of antibody response four weeks after the second HIV-MVA (HIVIS03 trial)**

Assay and antigen	Subtypes	Positive /total number tested (%)	Reference
<b>ELISA binding antibody</b>			Paper 1
gp120 CM243	AE	29/29 (100)	
gp140 <sub>96ZM651</sub>	C	29/29 (100)	
gp160 IIIB	B	26/29 (90)	Bakari et al [102]
<b>ADCC-GTL</b>			Paper 1
gp120 CM243	AE	24/28 (86)	
gp120 SF 162	B	21/28 (75)	
<b>ADCC-luciferase</b>			Paper 1
IMC <sub>CM235</sub>	AE	28/29 (97)	
IMC <sub>SF162</sub>	B	19/28 (68)	
<b>PBMC neutralization assay</b>			Bakari et al [102]
IMC CM235	AE	24/29 (83)	
IMC SF 162	B	21/29 (72)	
IMC BaL	B	9/29 (31)	
<b>TZM-bl pseudovirus assay</b>			Bakari et al [102] Paper I & II
CM 235, SF 162, Bal MW965.26	AE, B, B & C	0/29	
<b>gp70V1V2 ELISA</b>			Paper III
IgG gp70V1V2 A244	AE	28/29 (97)	
IgG gp70V1V2 CN54	C	14/29 (48)	
IgG gp70V1V2 Case A2	B	3/29 (10)	
IgG1 gp70V1V2 A244	AE	20/30 (67)	
IgG3 gp70V1V2 A244	AE	4/30 (13)	
IgG1 & IgG3 gp70V1V2 Consensus C	C	0/30	

ADCC-GTL, antibody-dependent cellular cytotoxicity-GranToxiLux assay; IMC, infectious molecular clone; ELISA, enzyme-linked immunosorbent assay; PBMC, peripheral blood mononuclear cell.

No ADCC activity was detected at baseline and two months after the first HIV-MVA boost in either of the two ADCC assays. After the second HIV-MVA vaccination, the frequency and magnitudes of ADCC-mediating antibody responses were higher to CRF01\_AE (86%, median titer 1841 in the ADCC-GTL assay and 97%, median titer 1076 in the ADCC-

Luciferase assay), compared to subtype B (75%, median titer 876 in the ADCC-GTL assay and 68%, median titer 715 in the ADCC-Luciferase assay) (Table 3).

The magnitude of ADCC-mediating antibody responses correlated well with the NAb titer against IMC CM235 (Figure 5a in paper 1). Higher titers of HIV-1 gp120 specific ADCC-mediating antibodies have been reported to correlate inversely with the rate of HIV disease progression, whereas significantly lower titers of ADCC antibodies were detected among HIV-1 infected rapid progressors compared to non rapid-progressors [151]. Moreover, studies conducted in non-human primates have shown that ADCC antibodies correlated with reduced acute viremia, following a mucosal challenge with pathogenic SIV in the vaccinated rhesus macaques [108,109]. In the RV144 trial, ADCC-mediating antibodies were reported to correlate inversely with reduced risk of HIV infection in vaccinees with low titers of IgA Env binding antibody [171].

We also assessed the binding antibody responses by ELISA at baseline and four weeks after the second HIV-MVA (Table 3). All 29 (100%) vaccinees had detectable antibodies both to CRF01\_AE gp120 (median titer 3200) and to HIV-1 subtype C gp140 (median titer 3200) after the second HIV-MVA vaccination. The binding antibody titers for these two subtypes were significantly higher than the previously reported titers against subtype B gp160, tested in the same vaccinees (Figure 6a in paper 1) [102]. A positive correlation was demonstrated between NAb titers against the CM235 IMC and the binding antibody titers to subtype C gp140. Furthermore, ADCC antibody titers against CM243 gp120 coated target cells correlated well with CM243 gp120 binding antibodies (Figure 6b & 6c in paper 1). A correlation between ADCC activity and anti-gp140 response has been demonstrated in SIV-infected rhesus macaques [155].

Overall, the three HIV-DNA priming and two HIV-MVA boosting immunizations generated potent functional antibody responses in a high proportion of healthy Tanzanian individuals. NK cells were responsible for the majority of the neutralizing activity observed in the IMC/PBMC neutralizing assay, supporting the observation of potent ADCC-mediating antibody responses.

## **5.2 Durability of immune responses after three HIV-DNA and two HIV-MVA immunizations and the effect of a late third HIV-MVA immunization (Paper II and III)**

In studies in paper II and III, we investigated the duration of immune responses in individuals primed with HIV-DNA and boosted with HIV-MVA in the HIVIS03 trial and the effect of an additional late third HIV-MVA immunization. Twenty volunteers who previously received three HIV-DNA and two HIV-MVA vaccinations were given a third HIV-MVA immunization three years after the second HIV-MVA boost, the HIVIS06 trial. The late third HIV-MVA vaccination was safe and well tolerated.

Binding Env antibody responses were measured in the 20 vaccinees who received a third HIV-MVA boost. Sera were tested at baseline, four weeks after the second HIV-MVA boost, at the time of the third HIV-MVA, three years after the second HIV-MVA and four weeks after the third HIV-MVA vaccination. The results are summarized in Table 4.

**Table 4. Frequency of immune responses before and after the third HIV-MVA vaccination (HIVIS06 trial)**

Assay and antigen	Subtypes	After 2 <sup>nd</sup> HIV-MVA	At the time of the 3 <sup>rd</sup> HIV-MVA	After 3 <sup>rd</sup> HIV-MVA
		Pos/total no. tested (%)	Pos/total no. tested (%)	Pos/total no. tested (%)
<b>ELISA binding antibody</b>				
gp120 CM243	AE	20/20 (100) <sup>a</sup>	18/20 (90)	20/20 (100) <sup>a</sup>
gp140 <sub>96ZM651</sub>	C	20/20 (100) <sup>a</sup>	18/20 (90)	20/20 (100) <sup>a</sup>
gp160 IIIB	B	17/19 (89) <sup>a</sup>	17/20 (85)	17/20 (85) <sup>a</sup>
<b>Neutralization</b>				
<b>PBMC assay</b> with p24 read out (SF162, CM244)	B, AE	0/20 <sup>a</sup>	ND	0/20 <sup>a</sup>
<b>TZM-bl assay</b> (SF162, MW965.26)	B	0/20 <sup>a</sup>	ND	0/20 <sup>a</sup>
<b>ADCC-GTL</b>				
gp120 CM243	AE	16/18 (88) <sup>a</sup>	16/19 (84)	18/19 (95) <sup>a</sup>
gp120 SF 162	B			
<b>ADCC-luciferase</b>				
IMC CM235	AE	18/19 (95) <sup>a</sup>	14/20 (70)	14/20 (70) <sup>a</sup>
IMC SF162	B	13/18 (72) <sup>a</sup>	9/19 (47)	14/19 (74) <sup>a</sup>
<b>gp70V1V2 ELISA</b>				
IgG gp70V1V2 A244	AE	19/19/ (100) <sup>a</sup>	15/20 (75)	19/20 (95) <sup>a</sup>
IgG gp70V1V2 CN54	C	9/19 (47) <sup>a</sup>	2/20 (10)	4/20 (20) <sup>a</sup>
IgG gp70V1V2 Case A2	B	3/19 (16) <sup>a</sup>	0/20	2/20 (10) <sup>a</sup>
IgG1 gp70V1V2 A244	AE	17/20 (85) <sup>a</sup>	5/20 (25)	13/20 (65) <sup>a</sup>
IgG3 gp70V1V2 A244	AE	3/20 (15) <sup>a</sup>	0/20	1/20 (5) <sup>a</sup>
IgG1 & IgG3 Consensus C	C	0/20 <sup>a</sup>	0/20	0/20 <sup>a</sup>
<b>ELISpot</b>				
Gag SMI	A	ND	13/19 (68)	17/20 (85) <sup>b</sup>
Gag CMDR	A	16/18 (89) <sup>b</sup>	12/19 (63)	18/20 (90) <sup>b</sup>
Env CMDR	E	16/18 (89) <sup>b</sup>	8/19 (42)	18/20 (90) <sup>b</sup>
Gag or Env		16/18 (89) <sup>b</sup>	14/19 (74)	19/20 (95) <sup>b</sup>
<b>Anti-vaccinia neutralization</b>				
Strain Elstree		19/20 (95) <sup>a</sup>	20/20 (100)	20/20 (100) <sup>a</sup>

ADCC-GTL, antibody-dependent cellular cytotoxicity-GranToxiLux assay; IMC, infectious molecular clone; ELISA, enzyme-linked immunosorbent assay; ND, Not done; PBMC, peripheral blood mononuclear cell; Pos, Positive; no, Number. <sup>a</sup>Testing performed 4 weeks after the HIV-MVA vaccination. <sup>b</sup>Testing performed 2 weeks after the HIV-MVA vaccination

A high proportion of the vaccinees showed durable binding antibody responses, 90% to subtype C gp140 and 85% to subtype B gp160, three years after the second HIV-MVA. In a



study by Goepfert et al, where volunteers were immunized twice with JS7 DNA and twice with MVA/HIV62B vaccines, binding antibodies to gp140 were elicited in 100% of the vaccinees two weeks after the final immunization. However, the responses decreased by 15.6% 24 weeks later [194], indicating a shorter durability of antibody responses compared to our study where the response declined by 5-10% three years after the second HIV-MVA immunization. In the same study by Goepfert et al, there was no decrease in the response rate of gp140 antibodies 24 weeks after three MVA/HIV62B vaccinations [194]. Following the third HIV-MVA vaccination in our study, the antibody response rate to subtype C gp140 increased to 100%. We also observed a significant increase in the magnitude of antibodies both to subtype C gp140 and subtype gp160 B after the third HIV-MVA (Figure 2 in paper II), showing that the antibody titer can be maintained by further HIV-MVA boosting. There was also a positive correlation of antibody titers against subtype C gp140 and subtype gp160 B.

ADCC-mediating antibody responses were measured using both gp120-coated target cells and IMC–infected target cells at three time points (Table 4). ADCC responses to subtype E CM243 gp120–coated cells were detected in 88% and to CM235 CRF01\_AE–infected cells in 95% of evaluable vaccinees after the second HIV- MVA. The majority of the vaccinees still had ADCC-mediating antibodies three years after the second HIV-MVA vaccination, 84% against CRF01\_AE gp120-coated cells (median titer 499) and 70% against CRF01\_AE–infected cells (median titer 239) indicating extraordinary durability. Following the third HIV-MVA vaccination the ADCC response to CRF01\_AE gp120–coated cells increased to 95% (median titer 683). Although the ADCC response rate against CM235 CRF01\_AE–infected cells remained the same after the third HIV-MVA, the magnitude increased significantly from a median titer of 239 to 578,  $p=0.004$ . After the second HIV-MVA, 72% of the vaccinees had ADCC response to SF162 subtype B–infected cells and 47% before the third HIV-MVA three years later, which increased to 74% after the third HIV-MVA boost. The magnitude increased significantly from a median titer of 204 to 681 ( $p = 0.0002$ ) nearly to the same level (median 571,  $p = 0.82$ ) as after the second HIV-MVA (Figure 3 in paper II). These findings demonstrate that priming with three HIV-DNA and boosting with two or more HIV-MVA, elicited durable and high titers of ADCC-mediating antibody responses in a significant proportion of vaccinees and that a late third HIV-MVA significantly boosted the ADCC responses. A lower frequency of ADCC activity was detected using 51Cr labeled CEM.Nkr coated cells, 40% against CM243 CRF01\_AE gp120 and 30% to MN subtype B gp120 in the RV158 trial among vaccinees who received three MVA-CMDR immunizations without HIV-DNA priming [195].

Several methods have been employed for the assessment of NAb in HIV vaccine trials using different types of target cells, culture conditions or signals to quantify the viral neutralization [190,193]. In the HIVIS06 study, NAb was measured using a PBMC assay with a p24-read out, as well as the TZM-bl based neutralizing assay. No NAb was detected in any of the two assays using sera collected from the vaccinees before and after the third HIV-MVA (Table 4). This finding is contrary to our previous report where a high frequency of NAb responses, up

to 83%, was demonstrated against CM235 CRF01\_AE using an IMC/PBMC assay [102]. The type of the PBMC neutralization assay used could explain the difference in neutralizing activity demonstrated. In the HIVIS06 study, we used a standard PBMC assay with a HIV p24 antigen read out, whereas the IMC/PBMC assay was used in the HIVIS03 study. In the IMC/PBMC assay the antibodies stay with the cells and virus for the entire four days duration of the assay, allowing other effector cells present in the PBMC cells, such as natural killer cells or monocytes, to affect the potential functional role, in contrast to the PBMC assay with a HIV p24 read out, where serum and virus are washed out after 24 hrs of incubation.

Antibodies to V1V2 of HIV Env were reported to correlate with reduced risk of HIV infection in the RV144 trial, the only trial that has shown an efficacy of 31.2 % [171]. In paper III, we analysed the V1V2-specific antibodies in plasma samples collected from 29 HIVIS03 vaccinees and 12 placebo recipients at baseline (V3) and four weeks after the second HIV-MVA (V21) and in 20 HIVIS06 vaccinees at the time of the third HIV-MVA, three years after the second HIV-MVA (V62) and four weeks after the third HIV-MVA (V64). Of the 29 vaccinees assessed, 28 (97%) had IgG binding antibodies to V1V2 of CRF01\_AE A244, 14 (48%) to subtype C CN54 and three (10%) to subtype B Case A2, four weeks after the second HIV-MVA vaccination (Table 3).

Of the 20 vaccinees who received an additional late third HIV-MVA immunization, all 19 (100%) evaluable vaccines exhibited V1V2 IgG antibodies to subtype E after the second HIV-MVA. The V1V2 antibody response showed an excellent durability. Three years after the second HIV-MVA, 75% of the vaccinees still had V1V2 antibody responses. In contrast, in the longitudinal follow-up study in the RV144 trial, Yates et al reported a high V1V2 IgG response rate of 97% to gp70 B Case A2 V1V2 at week 26 (2 weeks after the last immunization), which declined to 0% at week 156 (33 months after the last immunization). Similarly, the V1V2 IgG response against subtype C.1086 V1V2 tags was 97% and decreased to 8% at week 156 [173].

Following the third HIV-MVA immunization in our study, the V1V2 IgG response rate to CRF01\_AE A244 increased to 95%. The magnitude of V1V2-specific antibody against CRF01\_AE A244 increased significantly from a median titer of 400 at the three-year time point to a median of 1600,  $p < 0.0001$  after the third HIV-MVA, but not to the same level as after the second HIV-MVA (median titer 3200,  $p = 0.0257$ ) (Figure 1 in paper III). The response rate to V1V2 of subtype C CN54 declined from 47% four weeks after the second HIV-MVA to 10% three years later, but increased to 20% four weeks after the third HIV-MVA boost (Table 4). Antibody responses to V1V2 subtype B Case A2 were rare. None of the placebo recipients had antibodies to the three scaffolded gp70 V1V2 proteins.

Given that V1V2 IgG antibodies were detected in the majority of the vaccinees, we next tested V1V2 IgG subclass (IgG1 and IgG3) responses to subtype E A244 and subtype C Consensus C (Table 4). A high proportion of vaccinees (85%) had anti-V1V2 IgG1, four weeks after the second HIV-MVA, with three years durability in 25%. The response rate was boosted to 65% four weeks after the third HIV-MVA, a response rate similar to that seen after

the second HIV-MVA,  $p=0.273$ . The V1V2-specific IgG3 response rate was 15% after the second HIV-MVA and 5% after the third HIV-MVA. Likewise, the magnitude of anti-V1V2 IgG1 to subtype E A244 was also boosted significantly, from a median titer of 100 at the time of the third HIV-MVA to 200, four weeks after the third HIV-MVA,  $p=0.001$  (Figure 2 in paper III). In this study we observed higher responses of V1V2 IgG1 than V1V2 IgG3 and this reflects the overall level of IgG1 and IgG3 in the plasma. Similarly, V1V2 IgG1 responses were more frequently detected than V1V2 IgG3 responses in the RV144 trial [164]. Notably the IgG1 and IgG3 antibodies targeting the crown of the HIV Env V2 loop were more frequently elicited in the RV144 Thai trial than in the non-protective VAX003 trial [164]. The frequency and magnitude of V1V2 IgG3 responses were correlated with a decreased risk of HIV-1 infection in the RV144 trial [173]. In addition the rate of V1V2 IgG3 responses was higher in the RV144 trial than in the VAX003 trial [173]. Furthermore, vaccine-induced V1V2 IgG3 and IgG responses were not predictive of one another [173].

Antibodies to cyclic V2 peptides were also measured against subtype E A244 and consensus C using SPR/Biacore analysis at four time points (Figure 3 in paper III). The level of reactivity decreased significantly three years after the second HIV-MVA ( $p<0.0001$ ), and could be boosted by the third HIV-MVA immunization ( $p<0.0001$ ). None of the vaccinees had antibody reactivity to MN subtype B. No antibody reactivity was detected among placebo recipients. The Biacore data reported here are consistent with the ELISA findings.

This study of V1V2 antibodies had some limitations. V1V2 IgG2 and V1V2 IgG4 antibodies were not evaluated. It has been speculated that higher levels of V1V2 IgG2 and IgG4 antibodies may have interfered with the ADCC function leading to lack of protection in the VAX003 trial in contrast to the RV144 trial that demonstrated an efficacy of 31.2%. Furthermore, the lack of protection in the VAX003 trial has been reported to be associated with repeated immunizations of higher doses of vaccine antigen without adequate adjuvant signals [164], causing the IgG subclass switching.

In the HIVIS03/06 vaccinees, no correlation was demonstrated between the V1V2 specific antibodies and ADCC-mediating antibodies before and after the third HIV-MVA vaccination, suggesting that these antibodies target different epitopes (Table 4 in paper III). IgG antibodies specific to V1V2 have been reported to inhibit the viral entry, possibly by blocking the binding of V1V2 to the co-receptor CCR5 or to the integrin  $\alpha 4\beta 7$  receptor [196-198].

Durable cellular immune responses were also demonstrated in the HIVIS06 trial. A high proportion of vaccinees still had detectable IFN- $\gamma$  ELISpot responses three years after the second HIV-MVA vaccination, 63% to Gag-CMDR and 42% to Env-CMDR. Following the third HIV-MVA, the IFN- $\gamma$  ELISpot response increased to 95%, 90% to both Gag and Env-CMDR. The magnitude of responses to both Gag and Env declined significantly three years after the second HIV-MVA and was boosted significantly after the third HIV-MVA, to a level slightly lower than the level detected after the second HIV-MVA (Figures 4a & 4b in paper II). In a study where volunteers were given three doses of MVA-CMDR vaccination

without DNA priming, lower IFN- $\gamma$  ELISpot responses were reported, 30% to Gag and 60% to Env [195].

HIV-DNA priming in the HIVIS03 trial was administered either by the id or im route, using a needle free Biojector® device. After the second HIV-MVA, no significant difference in NAb was detected between HIV-DNA id-primed and im-primed vaccinees ( $p = 0.67$ ). Similarly, the ADCC-mediating antibody responses did not differ significantly between HIV-DNA id or im primed vaccinees at all testing time points, using both gp120 coated cells and IMC-infected target cells for the virus included. Interestingly, in the HIVIS06 study, we noted that the magnitude of the IFN- $\gamma$  ELISpot Env responses among the previously HIV-DNA id primed vaccinees was significantly higher at the three year time point and after the third HIV-MVA boost, than in the HIV-DNA im recipients, suggesting that id priming may be better than im priming for maintaining durable Env responses (Figure 4d in paper II).

The effect of vaccinia NAbS was also assessed before and after the HIV-MVA immunizations (Figure 5 in paper II). One volunteer had detectable vaccinia NAbS pre-vaccination. All vaccinees (100%) developed vaccinia-NAbS after HIV-MVA immunizations detectable at all the three-time points. In our study the presence of vaccinia NAbS did not affect the induction of humoral or cellular immune responses following a late third HIV-MVA. The finding reported here is consistent with our previous report, where the same vaccine regime was used [199]. Collectively, these findings suggest that a vaccinia vector-based vaccine can be used even in vaccinia pre-exposed individuals.

In summary, the HIV-DNA/MVA prime-boost regimen elicited potent humoral and cellular immune responses with extraordinary durability that persisted for more than three years. Furthermore, the third HIV-MVA vaccination significantly boosted the antibody and cellular immune responses.

### **5.3 Safety and immunogenicity of boosting with CN54rgp140/GLA-AF following HIV-DNA/MVA immunizations (Paper IV)**

This study was performed as an amendment to a phase IIa randomized placebo-controlled trial, in which healthy Tanzanian volunteers received three HIV-DNA immunizations or placebo followed by two HIV-MVA vaccinations or placebo (TaMoVac 01). Forty volunteers (35 vaccinees and 5 placebo recipients) were recruited to receive two CN54rgp140/GLA-AF immunizations, 31 to 70 weeks after the second HIV-MVA. The protein boost was safe and well tolerated, except in one volunteer with mild pre-existing hypoglycemia who developed severe hypoglycemia, an event that has not been reported in previous trials using the same protein vaccine.

Env-specific binding antibody responses were significantly boosted by the CN54rgp140/GLA-AF immunizations. Env protein that matched the CN54rgp140/GLA-AF immunogen was included in the analyses of binding antibodies. The binding IgG responses were analyzed against subtype C, B and CRF01\_AE Env four weeks after the second HIV-

MVA and four weeks after the first and second CN54rgp140/GLA-AF immunizations. Overall the response rate and titers were higher against subtype C CN54 rgp140 than against subtype B gp160 and CRF01\_AE gp120. After the second HIV-MVA vaccination, 97% of the vaccinees exhibited binding antibodies to subtype C CN54 rgp140. The frequency increased to 100% after the first and second CN54rgp140/GLA-AF vaccination. The magnitude increased significantly both after the first (median titer 8100) and second (median titer 24300) CN54rgp140/GLA-AF immunization compared to after the second HIV-MVA immunization (median titer 900)  $p < 0.0001$  (Figure 2 in paper IV). In the Thai RV144 trial in which individuals were primed with four ALVAC-HIV vCP1521 and boosted with two AIDSVAX B/E immunizations, delivered at the time of the two last ALVAC immunizations, high Env antibody response rates were demonstrated at 12 months, 98% to both CRF01\_AE gp120 A244 and subtype Bgp120 MN with geometric mean antibody titers (GMT) of 14558 and 31207, respectively [119]. In TaMoVac 01, the frequency of binding antibodies to subtype B gp160 was 87% after the second HIV-MVA vaccination. The response rate was further boosted by subtype C CN54rgp140/GLA-AF vaccination to 100% after the first and to 97% following the second protein boost. The median titer to subtype B gp160 increased from 400 after the second HIV-MVA, to 2700 after the two CN54rgp140/GLA-AF boost vaccinations. In addition, the antibody response rate to CRF01\_AE gp120 was 80% after the second CN54rgp140/GLA-AF boost, a significant increase, as compared to after the second HIV-MVA (49%),  $p = 0.012$ .

ADCC-mediating antibodies were measured using target cells infected with Env-IMC-LucR viruses, including CM235 CRF01\_AE, subtype B SF 162 and subtype C 1086. After the second HIV-MVA, 10/35 (29%) of the vaccinees demonstrated ADCC-mediating antibody responses to CM235 CRF01\_AE and 9/35 (25%) after the second CN54rgp140/GLA-AF immunization. The magnitude of the ADCC-mediating antibody responses against CM235 CRF01\_AE did not differ significantly after the second HIV-MVA (median titer 1801, range 201–4772) and after the second CN54rgp140/GLA-AF immunization (median titer 491, range 249–18140)  $p = 0.802$  (Figure 3 in paper IV). Only one of 35 vaccinees had ADCC-mediating antibodies to SF 162 subtype B and HIV 1086.c subtype C IMC after the second CN54rgp140/GLA-AF immunization (Table 5).

**Table 5: Comparison of immune responses elicited following immunization with two HIV-MVA, three HIV-MVA and two Env protein boosts in individuals primed with three HIV-DNA vaccinations.**

Assay and Antigen	Subtypes	HIVIS03/06 trial		TaMoVac01/rg140 trial	
		After two HIV-MVA n=29	After three HIV-MVA n=20	After two HIV-MVA n=35	After two Env Protein n=35
		Pos/total no. tested (%)	Pos/total no. tested (%)	Pos/total no. tested (%)	Pos/total no. tested (%)
<b>ELISA binding Ab</b>					
gp120 CM243	AE	29/29 (100)	20/20 (100)		
gp120 <sub>93TH975</sub>	AE			17/35 (49)	28/35 (69)
gp140 <sub>96ZM651</sub>	C	29/29 (100)	20/20 (100)	34/35 (97)	35/35 (100)
gp160 IIIB	B	26/29 (90)	17/20 (85)	25/30 (87)	28/29 (97)
<b>ADCC-GTL</b>					
gp120 CM243	AE	24/28 (86)			
gp120 SF 162	B	21/28 (75)			
<b>ADCC-luciferase</b>					
IMC CM235	AE	28/29 (97)	14/20 (70)	10/35 (29)	9/35 (28)
IMC SF162	B	19/28 (68)	14/19 (74)	2/35 (6)	1/35 (3)
IMC 1086	C			0/35	1/35 (3)
<b>IMC/PBMC neutralization assay</b>					
IMC CM235	AE	24/29 (83)		2/35 (6)	2/35 (6)
IMC SF 162	B	21/29 (72)		1/35 (3)	0/35
IMC GS015	C			7/35 (20)	11/35 (31)
IMC BaL	B	9/29 (31)			
<b>PBMC assay with p24 read out</b>	B, AE		0/20		
SF162, CM244					
<b>TZM-bl pseudovirus</b>					
CM235, SF162, BaL, MW965.26	AE, B, B, C	0/29	0/20		0/35
<b>ELISpot</b>					
Gag CMDR	A	23/29 (79)	18/20 (90)	26/33 (79)	17/35 (49)
Env CMDR	E	24/29 (83)	18/20 (90)	26/31 (84)	24/35 (69)
<b>LPA</b>					
CM235	AE	25/25 (100)			11/14 (79)
MN	B				12/14 (86)

ADCC-GTL, antibody-dependent cellular cytotoxicity-GranToxiLux assay; IMC, infectious molecular clone; ELISA, enzyme-linked immunosorbent assay; ND, Not done; PBMC, peripheral blood mononuclear cell; LPA, Lymphoproliferative assay. Pos, Positive; no, Number

In general, the functional antibody (ADCC and NAb) response rates in the TaMoVac 01 study were lower after the second HIV-MVA, as well as after the second CN54rgp140/GLA-AF vaccinations compared to the response rates reported in the HIVIS03/06 trials where volunteers were also primed with three HIV-DNA and boosted with two HIV-MVA immunizations (Table 5). The difference may be due to the interval between the HIV-DNA prime and HIV-MVA boosts, which was shorter in the TaMoVac 01 trial than in the HIVIS03/06 trial. In both trials, the HIV-DNA priming immunizations were given at 0, 1 and 3 months. However in the HIVIS03 trial, the HIV-MVA boosts were given at 9 and 21 months i.e. with an interval of 12 months between the first and second HIV-MVA boosts, whereas in the TaMoVac 01 trial the HIV-MVA boosts were given at 7.5 and 11.5 months i.e. with an interval of only 4 months. Longer intervals between priming and boosting have been reported to improve the induction of immune responses [200].

The impact of combined MVA and adjuvanted Env gp140 protein boost versus sequential administration of MVA/gp140 vaccine after DNA priming was recently reported by Joseph et al in a phase 1 clinical trial [122]. The overall immunization period was shorter in the combined immunization group. No significant impact was observed on the frequency and magnitude of Env-specific binding antibody responses to gp140 after combined vaccinations compared to sequential immunizations. However, the NAb response observed to the tier 1 virus was lower and the breadth of T cell responses tended to be lower when the vaccines were combined. This study employed a relatively short (4-8 weeks) interval between the immunizations and the authors hypothesize that this may have influenced the results [122].

We also assessed the IFN- $\gamma$  ELISpot and LPA responses in TaMoVac 01 vaccinees. The frequency and magnitude of IFN- $\gamma$  ELISpot responses to Env were significantly boosted following the second CN54rgp140/GLA-AF immunizations but not the responses to Gag-CMDR (Figure 4 in paper IV). A lymphocyte proliferation assay (LPA) was performed to further evaluate the vaccine-induced T-cell responses in 16 vaccinees from the MUHAS trial site. At the time of the first CN54rgp140/GLA-AF immunization, 8/14 (57%) of evaluable vaccinees had a proliferative response to subtype B MN, which increased non-significantly to 12/14 (86%) two weeks after the second CN54rgp140/GLA-AF. The response rate to CRF01\_AE CM235 was 9/14 (64%) at the time of the first CN54rgp140/GLA-AF, which increased to 11/14 (79%) two weeks after the second CN54rgp140/GLA-AF. The magnitude of the LPA response to subtype B MN increased significantly after the second rgp140/ GLA-AF immunization,  $p = 0.041$ .

In conclusion, two immunizations with subtype C gp140 Env protein adjuvanted in GLA-AF significantly boosted the Env specific binding antibody responses and Env-specific cellular immune responses, but not the functional antibody responses in vaccinees previously primed with HIV-DNA and HIV-MVA.

## 6 CONCLUSIONS, RECOMMENDATIONS AND FUTURE PLANS

The HIVIS03 and HIVIS06 studies showed that priming three times with HIV-DNA and boosting twice with a recombinant HIV-MVA vaccine induced potent HIV-specific ADCC-mediating and Env-binding antibody responses, including V1V2 antibodies, as well as cellular immune responses with excellent three-year durability. A late third HIV-MVA immunization given three years after the second HIV-MVA was well tolerated and significantly boosted both antibody and cellular HIV-specific immune responses, suggesting that immune responses could be maintained by regular boosting.

In study IV (the TaMovac 01 trial), we found a significant enhancement of Env binding antibody responses and Env-specific cell-mediated immune responses, after sequential boosting with CN54rgp140/GLA-AF protein of volunteers previously primed with three HIV-DNA and two HIV-MVA immunizations. However, overall, the immune responses, especially functional antibody responses, were not as frequent in the TaMoVac 01 trial vaccinees as in the HIVIS03/06 trial vaccinees, which may be due to the shorter interval between the HIV-MVA boosts in the TaMoVac 01 trial. This needs further study, and we recommend that the time interval between immunizations should be long enough to allow for maturation of the immune responses.

In our studies conducted in Tanzania and those conducted in Sweden using the same HIV-DNA vaccine for priming, immune responses were very rarely generated against HIV RT, which is expressed by one of the DNA plasmids. The findings suggest that, this plasmid could be excluded from the HIV-DNA vaccine.

Through the HIVIS03/06 and TaMoVac 01 clinical trials conducted in Tanzania, we have gained knowledge towards optimization of the immunization protocol for the HIV-DNA prime/ HIV-MVA boost vaccine regimen with and without gp140 protein. Based on the immunogenicity results obtained, the potential of the HIV-DNA /MVA prime boost vaccines regimen for the induction of potent and durable immune responses has been shown, and we therefore recommend this vaccine regimen as a potential avenue in HIV vaccine development. Nevertheless, further work is needed.

Following the HIV vaccine trials reported in this thesis, we were able to conduct a multicenter clinical trial involving two sites in Tanzania and one site in Mozambique, the phase II TaMoVac 02 trial that investigated the safety and immunogenicity of vaccine regimens comparing the use of id HIV-DNA prime alone or with id electroporation, and boosting with HIV-MVA alone or in combination with CN54rgp140/GLA-AF protein.

Preliminary findings showed that intradermal electroporation of HIV-DNA was well tolerated but did not potentiate priming by needle-free injection. Simplified HIV-DNA priming by one injection in a higher vaccine concentration, induced similar immune responses as compared to two injections with lower concentrations. The majority of the vaccinees developed subtype



C gp140-binding antibodies. The magnitude of gp140-specific binding antibodies was significantly higher after co-administration of rgp140/GLA-AF with HIV-MVA. The addition of Env protein to the HIV-MVA boost significantly increased the NAb response to homologous subtype C, but not to subtype AE pseudovirus homologous to HIV-MVA.

The capacity built which includes human resources training, strengthening of laboratory infrastructure and establishment of various techniques to evaluate vaccine-induced immune responses through the conduct of these trials in Tanzania have paved the way for possible future phase IIB and III HIV vaccine efficacy trials.

## 7 ACKNOWLEDGEMENTS

First and foremost I would like to thank the Almighty God, the father of glory for extending His grace, love and mercy to me throughout the time of my training. Indeed, it wasn't by power nor by might but by His Spirit.

I would like to express my sincere gratitude to everyone who contributed in one way or another to the progress and completion of my training. It's not possible to mention every one, but I would like specifically to thank my supervisors for their invaluable persistent support and expertise guidance throughout the entire time of my training.

Specifically, I would like to express my heartfelt gratitude to Prof. Charlotta Nilsson, my main supervisor for your guidance, all the great scientific discussions, encouragement and your countless support during the years of my training. Thank you very much for your enthusiasm to my training and for always believing in me. I have learnt a lot of things from you that helped to speed up the pace of my study.

I would like to extend sincere gratitude to Prof. Gunnel Biberfeld, my supervisor for your professional guidance and expertise, support and scientific ideas and discussions. Thank you for introducing me to the various scientific research laboratories where I learnt and acquired a lot of scientific skills and knowledge. Indeed, your commitment, supervision and passion towards my PhD training is untold. Thank you very much.

My profound appreciation goes to Prof. Eligius F. Lyamuya, my co-supervisor. Indeed there are not enough words to express my deep and sincere gratitude for your guidance, endless support and encouragement. You have been my supervisor since 2006, when I was doing my MMed at MUHAS, and you are the one who motivated me to join the PhD training. Thank you for giving me this wonderful opportunity and for always believing in me. I will forever be grateful for your tireless support.

My heartfelt gratitude goes to Prof. Said Aboud, my co-supervisor for your tireless guidance, tremendous and consistent support, supervision and encouragement throughout the study.

During my study I had an opportunity to be mentored and guided by Prof. Fred Mhalu. Thank you for your remarkable and tireless support, encouragement and professional advice.

I would like to extend my sincere appreciation to all the volunteers who participated in these studies, without you this work would not have been accomplished.

Sincere gratitude also goes to Prof. Muhammad Bakari, the Principal investigator HIVIS03/TaMoVac01 studies, Prof. Eric Sandström and Prof. Britta Wahren for your guidance, support and scientific advice. Thank you Prof. Mohameed Janabi and Dr. Eric Aris for advice and support.

I would like to express my heartfelt gratitude to Prof. Guido Ferrari from Duke University Medical Centre, Dr. Vicky Polonis and Dr. Mangala Rao from MHRP WRAIR and Prof. Christof Geldmacher from Munich University and their teams for training me in various laboratory techniques and data analysis.

In the course of my PhD training, I had an opportunity to discuss, share and learn a lot of issues related to my study from my colleagues at MUHAS and MNH. Specifically I would like to extend my gratitude to Dr. Patricia Munseri, Dr. Candida Moshiri, Dr. Matilda Ngarina and Dr. Edith Tarimo for your advice, support and encouragement.

I would also like to express my special thanks to Katarina Karlen, Teghesti Tecleab, Dr. Karina Godoy and Anita Östborn from the Public Health Agency of Sweden for laboratory training and logistic procedures during my studies.

I am grateful to Prof. Anna Mia Ekström and your wonderful team, Veronika Tirado, Galit Zeluf and Karin Båge from Department of Public Health Sciences/ Division of Global Health at KI for your support and assistance. Thank you for giving me special desk where I could comfortably sit and write my thesis. Special thank to Prof. Asli Kulane, for your encouragement and care.

Special thanks to the Head Department of Microbiology and Immunology at MUHAS, the previous and current Prof. Mecky Matee and Dr. Majigo Mtebe for your support and encouragement.

I am deeply grateful to MUHAS/ HIVIS03/06 and TaMoVac laboratory and clinical team; Emanuel Salala, Colman Mchau, Zakaria Mtulo, Haule Elionora, Salma Rashid, Salome Kihampa, Nasra Said, Scolastika Mahundi, Magdalena Kasya, Dr. Judica Mbwana, Viola Msangi and Andrea Sapula, Deus Buma, Mary Ngatoluwa, Tumaini Massawa, Dorothea Niima, Gladness Kiwelu, Lughano Kabadi, Tekla Mtomoni, for their technical support and commitment.

To my colleagues in the Department of Microbiology and Immunology at MUHAS; Prof. Samweli Massele, Dr. Sabrina Moyo, Dr. Joel Manyahi, Dr. Sima Rugabaramu, Dr. Frank Msafiri, Dr. Salim Salum, Lilian Nkinda, Upendo Kibwana and Grace Mutasa, thank you all for your support and encouragement.

I wish to express my gratitude to my fellow PhD students; Dr. Helga Naburi, Dr. Theodora Mbunda, Dr. Tumaini Nagu, Dr. Sabina Mugusi, Dr. Nelson Tembe, Edna Viegas and Hind Eliadarous. Thank you all for your invaluable support, comments and encouragement during my training.

Without a good shelter and a house to live while in Sweden, during my study, life would have been very challenging. Thus, I would like to specifically thank Gun Sandell for giving me a home while in Sweden. Thank you very much for your kindness, love and care.

My research training has been generously funded by The Swedish International Development Cooperation Agency (Sida). I would also like to extend my sincere gratitude to MUHAS management for granting me permission to undertake the PhD training.

Special thanks also to my wonderful parents, Baba Joachim Mkumbara and Mama Angela Shirima, my cousins and all my family members for your love, care and support.

Finally, my lovely husband Edwin Nyella and my beautiful daughter Divine, for your prayers, love, care, social and spiritual support, encouragement and tolerance for being away frequently, thank you so much.

## 8 REFERENCES

1. Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, et al. (1981) Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med 305: 1425-1431.
2. CDC (1981) Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men--New York City and California. MMWR Morb Mortal Wkly Rep 30: 305-308.
3. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, et al. (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 220: 868-871.
4. Gallo RC, Salahuddin SZ, Popovic M, Shearer GM, Kaplan M, et al. (1984) Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science 224: 500-503.
5. Clavel F, Guetard D, Brun-Vezinet F, Chamaret S, Rey MA, et al. (1986) Isolation of a new human retrovirus from West African patients with AIDS. Science 233: 343-346.
6. Clavel F (1987) HIV-2, the West African AIDS virus. AIDS 1: 135-140.
7. Sharp PM, Hahn BH (2011) Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med 1: a006841.
8. Coffin J, Haase A, Levy JA, Montagnier L, Oroszlan S, et al. (1986) What to call the AIDS virus? Nature 321: 10.
9. World Health Organization (2010) Priority interventions: HIV/AIDS prevention, treatment and care in the health sector. <http://apps.who.int/44418>.
10. Girard MP, Osmanov SK, Kieny MP (2006) A review of vaccine research and development: the human immunodeficiency virus (HIV). Vaccine 24: 4062-4081.
11. UNAIDS Fact sheet November 2016; Global HIV statistics. [http://www.unaids.org/sites/default/files/media\\_asset/UNAIDS\\_FactSheet\\_enpdf](http://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_enpdf).
12. AIDS, Global statistics 2015. <https://www.aids.gov/hiv-aids-basics/hiv-aids-101/global-statistics/>.
13. UNAIDS (2016) Prevention Gap Report.
14. AVERT, HIV and AIDS in Tanzania 2015. <https://www.avert.org/professionals/hiv-around-world/sub-saharan-africa/tanzania>.
15. Tanzania Ministry of Health (2014) Global AIDS Response Country Progress Report.
16. Tanzania Commission for AIDS (2013) '2011-12' Tanzania HIV/AIDS and Malaria Indicator Survey
17. Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg CM, et al. (1999) Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature 397: 436-441.
18. Sharp PM, Robertson DL, Hahn BH (1995) Cross-species transmission and recombination of 'AIDS' viruses. Philos Trans R Soc Lond B Biol Sci 349: 41-47.

19. Sierra S, Kupfer B, Kaiser R (2005) Basics of the virology of HIV-1 and its replication. *J Clin Virol* 34: 233-244.
20. Murray P, Resenthal K, Pfaller M (2009) Medical Microbiology 6th Edition. p627 -641.
21. Fanales-Belasio E, Raimondo M, Suligoi B, Butto S (2010) HIV virology and pathogenetic mechanisms of infection: a brief overview. *Ann Ist Super Sanita* 46: 5-14.
22. Clapham PR, McKnight A (2002) Cell surface receptors, virus entry and tropism of primate lentiviruses. *J Gen Virol* 83: 1809-1829.
23. Coffin JM (1992) Genetic diversity and evolution of retroviruses. *Curr Top Microbiol Immunol* 176: 143-164.
24. Peeters M, Toure-Kane C, Nkengasong JN (2003) Genetic diversity of HIV in Africa: impact on diagnosis, treatment, vaccine development and trials. *AIDS* 17: 2547-2560.
25. Lynch RM, Shen T, Gnanakaran S, Derdeyn CA (2009) Appreciating HIV type 1 diversity: subtype differences in Env. *AIDS Res Hum Retroviruses* 25: 237-248.
26. Simon F, Mauclore P, Roques P, Loussert-Ajaka I, Muller-Trutwin MC, et al. (1998) Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. *Nat Med* 4: 1032-1037.
27. Ayoub A, Souquieres S, Njinku B, Martin PM, Muller-Trutwin MC, et al. (2000) HIV-1 group N among HIV-1-seropositive individuals in Cameroon. *AIDS* 14: 2623-2625.
28. Buonaguro L, Tornesello ML, Buonaguro FM (2007) Human immunodeficiency virus type 1 subtype distribution in the worldwide epidemic: pathogenetic and therapeutic implications. *J Virol* 81: 10209-10219.
29. Osmanov S, Pattou C, Walker N, Schwardlander B, Esparza J (2002) Estimated global distribution and regional spread of HIV-1 genetic subtypes in the year 2000. *J Acquir Immune Defic Syndr* 29: 184-190.
30. Tatt ID, Barlow KL, Nicoll A, Clewley JP (2001) The public health significance of HIV-1 subtypes. *Aids* 15: S59-S71.
31. Lyamuya E, Olausson-Hansson E, Albert J, Mhalu F, Biberfeld G (2000) Evaluation of a prototype Amplicor PCR assay for detection of human immunodeficiency virus type 1 DNA in blood samples from Tanzanian adults infected with HIV-1 subtypes A, C and D. *J Clin Virol* 17: 57-63.
32. Arroyo MA, Hoelscher M, Sanders-Buell E, Herlinger KH, Samky E, et al. (2004) HIV type 1 subtypes among blood donors in the Mbeya region of southwest Tanzania. *AIDS Res Hum Retroviruses* 20: 895-901.
33. Nyombi BM, Kristiansen KI, Bjune G, Muller F, Holm-Hansen C (2008) Diversity of human immunodeficiency virus type 1 subtypes in Kagera and Kilimanjaro regions, Tanzania. *AIDS Res Hum Retroviruses* 24: 761-769.
34. Lau KA, Wong JJ (2013) Current Trends of HIV Recombination Worldwide. *Infect Dis Rep* 5: e4.
35. Pantaleo G, Fauci AS (1995) New concepts in the immunopathogenesis of HIV infection. *Annu Rev Immunol* 13: 487-512.

36. Pantaleo G, Fauci AS (1996) Immunopathogenesis of HIV infection. *Annu Rev Microbiol* 50: 825-854.
37. Feinberg MB (1996) Changing the natural history of HIV disease. *Lancet* 348: 239-246.
38. CASCADE EU Concerted Action (2000) Time from HIV-1 seroconversion to AIDS and death before widespread use of highly-active antiretroviral therapy: a collaborative re-analysis. Collaborative Group on AIDS Incubation and HIV Survival including the CASCADE EU Concerted Action. Concerted Action on SeroConversion to AIDS and Death in Europe. *Lancet* 355: 1131-1137.
39. Morgan D, Mahe C, Mayanja B, Okongo JM, Lubega R, et al. (2002) HIV-1 infection in rural Africa: is there a difference in median time to AIDS and survival compared with that in industrialized countries? *AIDS* 16: 597-603.
40. Jaffar S, Grant AD, Whitworth J, Smith PG, Whittle H (2004) The natural history of HIV-1 and HIV-2 infections in adults in Africa: a literature review. *Bulletin of the World Health Organization* 82: 462-469.
41. Bakari M, Urassa W, Pallangyo K, Swai A, Mhalu F, et al. (2004) The natural course of disease following HIV-1 infection in dar es salaam, Tanzania: a study among hotel workers relating clinical events to CD4 T-lymphocyte counts. *Scand J Infect Dis* 36: 466-473.
42. Tindall B, Cooper DA (1991) Primary HIV infection: host responses and intervention strategies. *AIDS* 5: 1-14.
43. Holmes CB, Losina E, Walensky RP, Yazdanpanah Y, Freedberg KA (2003) Review of human immunodeficiency virus type 1-related opportunistic infections in sub-Saharan Africa. *Clin Infect Dis* 36: 652-662.
44. Murray JF (2005) Pulmonary complications of HIV-1 infection among adults living in Sub-Saharan Africa. *Int J Tuberc Lung Dis* 9: 826-835.
45. Charles W, Harrington W (2005) AIDS and associated malignancies. *Cell research* 15: 947-952.
46. Kanki PJ, Hamel DJ, Sankale JL, Hsieh C, Thior I, et al. (1999) Human immunodeficiency virus type 1 subtypes differ in disease progression. *J Infect Dis* 179: 68-73.
47. Vasan A, Renjifo B, Hertzmark E, Chaplin B, Msamanga G, et al. (2006) Different rates of disease progression of HIV type 1 infection in Tanzania based on infecting subtype. *Clin Infect Dis* 42: 843-852.
48. Kiwanuka N, Laeyendecker O, Robb M, Kigozi G, Arroyo M, et al. (2008) Effect of human immunodeficiency virus Type 1 (HIV-1) subtype on disease progression in persons from Rakai, Uganda, with incident HIV-1 infection. *J Infect Dis* 197: 707-713.
49. Kaleebu P, French N, Mahe C, Yirell D, Watera C, et al. (2002) Effect of human immunodeficiency virus (HIV) type 1 envelope subtypes A and D on disease progression in a large cohort of HIV-1-positive persons in Uganda. *J Infect Dis* 185: 1244-1250.
50. Baeten JM, Chohan B, Lavreys L, Chohan V, McClelland RS, et al. (2007) HIV-1 subtype D infection is associated with faster disease progression than subtype A in spite of similar plasma HIV-1 loads. *J Infect Dis* 195: 1177-1180.

51. Alaeus A, Lidman K, Bjorkman A, Giesecke J, Albert J (1999) Similar rate of disease progression among individuals infected with HIV-1 genetic subtypes A-D. *AIDS* 13: 901-907.
52. Kharsany AB, Karim QA (2016) HIV Infection and AIDS in Sub-Saharan Africa: Current Status, Challenges and Opportunities. *Open AIDS J* 10: 34-48.
53. Gouws E, Cuchi P (2012) Focusing the HIV response through estimating the major modes of HIV transmission: a multi-country analysis. *Sex Transm Infect* 88 Suppl 2: i76-85.
54. Shahmanesh M, Patel V, Mabey D, Cowan F (2008) Effectiveness of interventions for the prevention of HIV and other sexually transmitted infections in female sex workers in resource poor setting: a systematic review. *Trop Med Int Health* 13: 659-679.
55. Douek DC, Roederer M, Koup RA (2009) Emerging concepts in the immunopathogenesis of AIDS. *Annu Rev Med* 60: 471-484.
56. Cilliers T, Nhlapo J, Coetzer M, Orlovic D, Ketas T, et al. (2003) The CCR5 and CXCR4 coreceptors are both used by human immunodeficiency virus type 1 primary isolates from subtype C. *J Virol* 77: 4449-4456.
57. Fearon M (2005) The laboratory diagnosis of HIV infections. *Can J Infect Dis Med Microbiol* 16: 26-30.
58. CDC. Laboratory testing for the diagnosis of HIV infection 2014: Updated recommendation. <https://www.cdc.gov/hiv/testing/index.html>.
59. Hans R, Marwaha N (2014) Nucleic acid testing-benefits and constraints. *Asian J Transfus Sci* 8: 2-3.
60. Wittek M, Sturmer M, Doerr HW, Berger A (2007) Molecular assays for monitoring HIV infection and antiretroviral therapy. *Expert Rev Mol Diagn* 7: 237-246.
61. Rouet F, Ekouevi DK, Chaix ML, Burgard M, Inwoley A, et al. (2005) Transfer and evaluation of an automated, low-cost real-time reverse transcription-PCR test for diagnosis and monitoring of human immunodeficiency virus type 1 infection in a West African resource-limited setting. *J Clin Microbiol* 43: 2709-2717.
62. Thompson MR, Kaminski JJ, Kurt-Jones EA, Fitzgerald KA (2011) Pattern recognition receptors and the innate immune response to viral infection. *Viruses* 3: 920-940.
63. Kumagai Y, Takeuchi O, Akira S (2008) Pathogen recognition by innate receptors. *J Infect Chemother* 14: 86-92.
64. Loo YM, Gale M, Jr. (2007) Viral regulation and evasion of the host response. *Curr Top Microbiol Immunol* 316: 295-313.
65. Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C (2004) Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 303: 1529-1531.
66. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, et al. (2004) Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science* 303: 1526-1529.
67. Beignon AS, McKenna K, Skoberne M, Manches O, DaSilva I, et al. (2005) Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor-viral RNA interactions. *J Clin Invest* 115: 3265-3275.



68. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP (1999) Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev Immunol* 17: 189-220.
69. Zamai L, Ahmad M, Bennett IM, Azzoni L, Alnemri ES, et al. (1998) Natural killer (NK) cell-mediated cytotoxicity: differential use of TRAIL and Fas ligand by immature and mature primary human NK cells. *J Exp Med* 188: 2375-2380.
70. Maghazachi AA (2010) Role of chemokines in the biology of natural killer cells. *Curr Top Microbiol Immunol* 341: 37-58.
71. Robertson MJ (2002) Role of chemokines in the biology of natural killer cells. *J Leukoc Biol* 71: 173-183.
72. Abbas A, Lichtman, AH.; Pillai S (2015) *Cellular and Molecular Immunology* eighth Edition.
73. Trapani JA, Smyth MJ (2002) Functional significance of the perforin/granzyme cell death pathway. *Nat Rev Immunol* 2: 735-747.
74. Tomaras GD, Haynes BF (2009) HIV-1-specific antibody responses during acute and chronic HIV-1 infection. *Curr Opin HIV AIDS* 4: 373-379.
75. Tomaras GD, Yates NL, Liu P, Qin L, Fouda GG, et al. (2008) Initial B-cell responses to transmitted human immunodeficiency virus type 1: virion-binding immunoglobulin M (IgM) and IgG antibodies followed by plasma anti-gp41 antibodies with ineffective control of initial viremia. *J Virol* 82: 12449-12463.
76. Overbaugh J, Morris L (2012) The Antibody Response against HIV-1. *Cold Spring Harb Perspect Med* 2: a007039.
77. Doria-Rose NA, Klein RM, Daniels MG, O'Dell S, Nason M, et al. (2010) Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. *J Virol* 84: 1631-1636.
78. Pantophlet R, Burton DR (2006) GP120: target for neutralizing HIV-1 antibodies. *Annu Rev Immunol* 24: 739-769.
79. Gray ES, Moore PL, Choge IA, Decker JM, Bibollet-Ruche F, et al. (2007) Neutralizing antibody responses in acute human immunodeficiency virus type 1 subtype C infection. *J Virol* 81: 6187-6196.
80. Mascola JR, Montefiori DC (2010) The role of antibodies in HIV vaccines. *Annu Rev Immunol* 28: 413-444.
81. Wei X, Decker JM, Wang S, Hui H, Kappes JC, et al. (2003) Antibody neutralization and escape by HIV-1. *Nature* 422: 307-312.
82. Wyatt R, Sodroski J (1998) The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. *Science* 280: 1884-1888.
83. Simek MD, Rida W, Priddy FH, Pung P, Carrow E, et al. (2009) Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. *J Virol* 83: 7337-7348.
84. Stamatatos L, Morris L, Burton DR, Mascola JR (2009) Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine? *Nat Med* 15: 866-870.

85. Dhillon AK, Donners H, Pantophlet R, Johnson WE, Decker JM, et al. (2007) Dissecting the neutralizing antibody specificities of broadly neutralizing sera from human immunodeficiency virus type 1-infected donors. *J Virol* 81: 6548-6562.
86. Sather DN, Armann J, Ching LK, Mavrantoni A, Sellhorn G, et al. (2009) Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. *J Virol* 83: 757-769.
87. Robinson HL (2013) Non-neutralizing antibodies in prevention of HIV infection. *Expert Opin Biol Ther* 13: 197-207.
88. Huber M, Trkola A (2007) Humoral immunity to HIV-1: neutralization and beyond. *J Intern Med* 262: 5-25.
89. Sandberg JK, Fast NM, Nixon DF (2001) Functional heterogeneity of cytokines and cytolytic effector molecules in human CD8<sup>+</sup> T lymphocytes. *J Immunol* 167: 181-187.
90. Voskoboinik I, Smyth MJ, Trapani JA (2006) Perforin-mediated target-cell death and immune homeostasis. *Nat Rev Immunol* 6: 940-952.
91. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, et al. (1995) Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8<sup>+</sup> T cells. *Science* 270: 1811-1815.
92. Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, et al. (1997) Vigorous HIV-1-specific CD4<sup>+</sup> T cell responses associated with control of viremia. *Science* 278: 1447-1450.
93. Norris PJ, Rosenberg ES (2001) Cellular immune response to human immunodeficiency virus. *AIDS* 15 Suppl 2: S16-21.
94. Soghoian DZ, Jessen H, Flanders M, Sierra-Davidson K, Cutler S, et al. (2012) HIV-specific cytolytic CD4 T cell responses during acute HIV infection predict disease outcome. *Sci Transl Med* 4: 123ra125.
95. Johnson S, Eller M, Teigler JE, Maloveste SM, Schultz BT, et al. (2015) Cooperativity of HIV-Specific Cytolytic CD4 T Cells and CD8 T Cells in Control of HIV Viremia. *J Virol* 89: 7494-7505.
96. Smith MK, Rutstein SE, Powers KA, Fidler S, Miller WC, et al. (2013) The detection and management of early HIV infection: a clinical and public health emergency. *J Acquir Immune Defic Syndr* 63 Suppl 2: S187-199.
97. Auvert B, Taljaard D, Lagarde E, Sobngwi-Tambekou J, Sitta R, et al. (2005) Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial. *PLoS Med* 2: e298.
98. Mepham SO, Bland RM, Newell ML (2011) Prevention of mother-to-child transmission of HIV in resource-rich and -poor settings. *BJOG* 118: 202-218.
99. Young TN, Arens FJ, Kennedy GE, Laurie JW, Rutherford G (2007) Antiretroviral post-exposure prophylaxis (PEP) for occupational HIV exposure. *Cochrane Database Syst Rev*: CD002835.
100. Roehr B (2011) Tenofovir works as pre-exposure prophylaxis against HIV, two studies confirm. *BMJ* 343: d4540.

101. Thorner AR, Lemckert AA, Goudsmit J, Lynch DM, Ewald BA, et al. (2006) Immunogenicity of heterologous recombinant adenovirus prime-boost vaccine regimens is enhanced by circumventing vector cross-reactivity. *J Virol* 80: 12009-12016.
102. Bakari M, Aboud S, Nilsson C, Francis J, Buma D, et al. (2011) Broad and potent immune responses to a low dose intradermal HIV-1 DNA boosted with HIV-1 recombinant MVA among healthy adults in Tanzania. *Vaccine* 29: 8417-8428.
103. Sandstrom E, Nilsson C, Hejdeman B, Brave A, Bratt G, et al. (2008) Broad immunogenicity of a multigene, multiclade HIV-1 DNA vaccine boosted with heterologous HIV-1 recombinant modified vaccinia virus Ankara. *J Infect Dis* 198: 1482-1490.
104. Harari A, Bart PA, Stohr W, Tapia G, Garcia M, et al. (2008) An HIV-1 clade C DNA prime, NYVAC boost vaccine regimen induces reliable, polyfunctional, and long-lasting T cell responses. *J Exp Med* 205: 63-77.
105. Koup RA, Roederer M, Lamoreaux L, Fischer J, Novik L, et al. (2010) Priming immunization with DNA augments immunogenicity of recombinant adenoviral vectors for both HIV-1 specific antibody and T-cell responses. *PLoS One* 5: e9015.
106. Kibuuka H, Kimutai R, Maboko L, Sawe F, Schunk MS, et al. (2010) A phase 1/2 study of a multiclade HIV-1 DNA plasmid prime and recombinant adenovirus serotype 5 boost vaccine in HIV-Uninfected East Africans (RV 172). *J Infect Dis* 201: 600-607.
107. Alpert MD, Harvey JD, Lauer WA, Reeves RK, Piatak M, Jr., et al. (2012) ADCC develops over time during persistent infection with live-attenuated SIV and is associated with complete protection against SIV(mac)251 challenge. *PLoS Pathog* 8: e1002890.
108. Gomez-Roman VR, Patterson LJ, Venzon D, Liewehr D, Aldrich K, et al. (2005) Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIVmac251. *J Immunol* 174: 2185-2189.
109. Hidajat R, Xiao P, Zhou Q, Venzon D, Summers LE, et al. (2009) Correlation of vaccine-elicited systemic and mucosal nonneutralizing antibody activities with reduced acute viremia following intrarectal simian immunodeficiency virus SIVmac251 challenge of rhesus macaques. *J Virol* 83: 791-801.
110. Shacklett BL, Shaw KE, Adamson LA, Wilkens DT, Cox CA, et al. (2002) Live, attenuated simian immunodeficiency virus SIVmac-M4, with point mutations in the Env transmembrane protein intracytoplasmic domain, provides partial protection from mucosal challenge with pathogenic SIVmac251. *J Virol* 76: 11365-11378.
111. Excler JL, Plotkin S (1997) The prime-boost concept applied to HIV preventive vaccines. *AIDS* 11 Suppl A: S127-137.
112. Ramshaw IA, Ramsay AJ (2000) The prime-boost strategy: exciting prospects for improved vaccination. *Immunol Today* 21: 163-165.
113. Hurwitz JL, Zhan X, Brown SA, Bonsignori M, Stambas J, et al. (2007) A Multi-Vector, Multi-Envelope HIV-1 Vaccine. *J Pediatr Pharmacol Ther* 12: 68-76.
114. Amara RR, Villinger F, Altman JD, Lydy SL, O'Neil SP, et al. (2001) Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. *Science* 292: 69-74.

115. Hanke T, Samuel RV, Blanchard TJ, Neumann VC, Allen TM, et al. (1999) Effective induction of simian immunodeficiency virus-specific cytotoxic T lymphocytes in macaques by using a multiepitope gene and DNA prime-modified vaccinia virus Ankara boost vaccination regimen. *J Virol* 73: 7524-7532.
116. Kent SJ, Zhao A, Best SJ, Chandler JD, Boyle DB, et al. (1998) Enhanced T-cell immunogenicity and protective efficacy of a human immunodeficiency virus type 1 vaccine regimen consisting of consecutive priming with DNA and boosting with recombinant fowlpox virus. *J Virol* 72: 10180-10188.
117. Shiver JW, Fu TM, Chen L, Casimiro DR, Davies ME, et al. (2002) Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature* 415: 331-335.
118. Paris RM, Kim JH, Robb ML, Michael NL (2010) Prime-boost immunization with poxvirus or adenovirus vectors as a strategy to develop a protective vaccine for HIV-1. *Expert Rev Vaccines* 9: 1055-1069.
119. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, et al. (2009) Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* 361: 2209-2220.
120. Lisziewicz J, Calarota SA, Lori F (2007) The potential of topical DNA vaccines adjuvanted by cytokines. *Expert Opin Biol Ther* 7: 1563-1574.
121. Joachim A, Bauer A, Joseph S, Geldmacher C, Munseri PJ, et al. (2016) Boosting with Subtype C CN54rgp140 Protein Adjuvanted with Glucopyranosyl Lipid Adjuvant after Priming with HIV-DNA and HIV-MVA Is Safe and Enhances Immune Responses: A Phase I Trial. *PLoS One* 11: e0155702.
122. Joseph S, Quinn K, Greenwood A, Cope AV, McKay PF, et al. (2017) A Comparative Phase I Study of Combination, Homologous Subtype-C DNA, MVA, and Env gp140 Protein/Adjuvant HIV Vaccines in Two Immunization Regimes. *Front Immunol* 8: 149.
123. Mascola JR, Stiegler G, VanCott TC, Katinger H, Carpenter CB, et al. (2000) Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. *Nat Med* 6: 207-210.
124. Mascola JR, Lewis MG, Stiegler G, Harris D, VanCott TC, et al. (1999) Protection of Macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. *J Virol* 73: 4009-4018.
125. Barouch DH, Liu J, Li H, Maxfield LF, Abbink P, et al. (2012) Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. *Nature* 482: 89-93.
126. Hessel AJ, Rakasz EG, Tehrani DM, Huber M, Weisgrau KL, et al. (2010) Broadly neutralizing monoclonal antibodies 2F5 and 4E10 directed against the human immunodeficiency virus type 1 gp41 membrane-proximal external region protect against mucosal challenge by simian-human immunodeficiency virus SHIVBa-L. *J Virol* 84: 1302-1313.
127. Nishimura Y, Igarashi T, Haigwood NL, Sadjadpour R, Donau OK, et al. (2003) Transfer of neutralizing IgG to macaques 6 h but not 24 h after SHIV infection confers sterilizing protection: implications for HIV-1 vaccine development. *Proc Natl Acad Sci U S A* 100: 15131-15136.

128. Haigwood NL, Watson A, Sutton WF, McClure J, Lewis A, et al. (1996) Passive immune globulin therapy in the SIV/maaque model: early intervention can alter disease profile. *Immunol Lett* 51: 107-114.
129. Lewis MG, Elkins WR, McCutchan FE, Benveniste RE, Lai CY, et al. (1993) Passively transferred antibodies directed against conserved regions of SIV envelope protect macaques from SIV infection. *Vaccine* 11: 1347-1355.
130. Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, et al. (1999) Control of viremia in simian immunodeficiency virus infection by CD8<sup>+</sup> lymphocytes. *Science* 283: 857-860.
131. Wilson NA, Reed J, Napoe GS, Piaskowski S, Szymanski A, et al. (2006) Vaccine-induced cellular immune responses reduce plasma viral concentrations after repeated low-dose challenge with pathogenic simian immunodeficiency virus SIVmac239. *J Virol* 80: 5875-5885.
132. Betts MR, Nason MC, West SM, De Rosa SC, Migueles SA, et al. (2006) HIV nonprogressors preferentially maintain highly functional HIV-specific CD8<sup>+</sup> T cells. *Blood* 107: 4781-4789.
133. Pantaleo G, Koup RA (2004) Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. *Nat Med* 10: 806-810.
134. Letvin NL, Schmitz JE, Jordan HL, Seth A, Hirsch VM, et al. (1999) Cytotoxic T lymphocytes specific for the simian immunodeficiency virus. *Immunol Rev* 170: 127-134.
135. Jin X, Bauer DE, Tuttleton SE, Lewin S, Gettie A, et al. (1999) Dramatic rise in plasma viremia after CD8<sup>+</sup> T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med* 189: 991-998.
136. Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB (1994) Virus-specific CD8<sup>+</sup> cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J Virol* 68: 6103-6110.
137. Pontesilli O, Klein MR, Kerkhof-Garde SR, Pakker NG, de Wolf F, et al. (1998) Longitudinal analysis of human immunodeficiency virus type 1-specific cytotoxic T lymphocyte responses: a predominant gag-specific response is associated with nonprogressive infection. *J Infect Dis* 178: 1008-1018.
138. Beretta A, Furci L, Burastero S, Cosma A, Dinelli ME, et al. (1996) HIV-1-specific immunity in persistently seronegative individuals at high risk for HIV infection. *Immunol Lett* 51: 39-43.
139. Rowland-Jones S, Sutton J, Ariyoshi K, Dong T, Gotch F, et al. (1995) HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. *Nat Med* 1: 59-64.
140. Kaul R, Rowland-Jones SL, Kimani J, Fowke K, Dong T, et al. (2001) New insights into HIV-1 specific cytotoxic T-lymphocyte responses in exposed, persistently seronegative Kenyan sex workers. *Immunol Lett* 79: 3-13.
141. Clerici M, Giorgi JV, Chou CC, Gudeman VK, Zack JA, et al. (1992) Cell-mediated immune response to human immunodeficiency virus (HIV) type 1 in seronegative homosexual men with recent sexual exposure to HIV-1. *J Infect Dis* 165: 1012-1019.

142. Plummer FA, Ball TB, Kimani J, Fowke KR (1999) Resistance to HIV-1 infection among highly exposed sex workers in Nairobi: what mediates protection and why does it develop? *Immunol Lett* 66: 27-34.
143. Fowke KR, Nagelkerke NJ, Kimani J, Simonsen JN, Anzala AO, et al. (1996) Resistance to HIV-1 infection among persistently seronegative prostitutes in Nairobi, Kenya. *Lancet* 348: 1347-1351.
144. Trivedi HN, Plummer FA, Anzala AO, Njagi E, Bwayo JJ, et al. (2001) Resistance to HIV-1 infection among African sex workers is associated with global hyporesponsiveness in interleukin 4 production. *FASEB J* 15: 1795-1797.
145. Goh WC, Markee J, Akridge RE, Meldorf M, Musey L, et al. (1999) Protection against human immunodeficiency virus type 1 infection in persons with repeated exposure: evidence for T cell immunity in the absence of inherited CCR5 coreceptor defects. *J Infect Dis* 179: 548-557.
146. Ballan WM, Vu BA, Long BR, Loo CP, Michaelsson J, et al. (2007) Natural killer cells in perinatally HIV-1-infected children exhibit less degranulation compared to HIV-1-exposed uninfected children and their expression of KIR2DL3, NKG2C, and NKp46 correlates with disease severity. *J Immunol* 179: 3362-3370.
147. Ravet S, Scott-Algara D, Bonnet E, Tran HK, Tran T, et al. (2007) Distinctive NK-cell receptor repertoires sustain high-level constitutive NK-cell activation in HIV-exposed uninfected individuals. *Blood* 109: 4296-4305.
148. Tomescu C, Duh FM, Lanier MA, Kapalko A, Mounzer KC, et al. (2010) Increased plasmacytoid dendritic cell maturation and natural killer cell activation in HIV-1 exposed, uninfected intravenous drug users. *AIDS* 24: 2151-2160.
149. Tomescu C, Abdulhaqq S, Montaner LJ (2011) Evidence for the innate immune response as a correlate of protection in human immunodeficiency virus (HIV)-1 highly exposed seronegative subjects (HESN). *Clin Exp Immunol* 164: 158-169.
150. Mazzoli S, Trabattoni D, Lo Caputo S, Piconi S, Ble C, et al. (1997) HIV-specific mucosal and cellular immunity in HIV-seronegative partners of HIV-seropositive individuals. *Nat Med* 3: 1250-1257.
151. Baum LL, Cassutt KJ, Knigge K, Khattri R, Margolick J, et al. (1996) HIV-1 gp120-specific antibody-dependent cell-mediated cytotoxicity correlates with rate of disease progression. *J Immunol* 157: 2168-2173.
152. Wren LH, Chung AW, Isitman G, Kelleher AD, Parsons MS, et al. (2013) Specific antibody-dependent cellular cytotoxicity responses associated with slow progression of HIV infection. *Immunology* 138: 116-123.
153. Lambotte O, Ferrari G, Moog C, Yates NL, Liao HX, et al. (2009) Heterogeneous neutralizing antibody and antibody-dependent cell cytotoxicity responses in HIV-1 elite controllers. *AIDS* 23: 897-906.
154. Lambotte O, Pollara J, Boufassa F, Moog C, Venet A, et al. (2013) High antibody-dependent cellular cytotoxicity responses are correlated with strong CD8 T cell viral suppressive activity but not with B57 status in HIV-1 elite controllers. *PLoS One* 8: e74855.
155. Sun Y, Asmal M, Lane S, Permar SR, Schmidt SD, et al. (2011) Antibody-dependent cell-mediated cytotoxicity in simian immunodeficiency virus-infected rhesus monkeys. *J Virol* 85: 6906-6912.

156. Ljunggren K, Moschese V, Broliden PA, Giaquinto C, Quinti I, et al. (1990) Antibodies mediating cellular cytotoxicity and neutralization correlate with a better clinical stage in children born to human immunodeficiency virus-infected mothers. *J Infect Dis* 161: 198-202.
157. Roussilhon C, Oeuvray C, Muller-Graf C, Tall A, Rogier C, et al. (2007) Long-term clinical protection from falciparum malaria is strongly associated with IgG3 antibodies to merozoite surface protein 3. *PLoS Med* 4: e320.
158. Kam YW, Simarmata D, Chow A, Her Z, Teng TS, et al. (2012) Early appearance of neutralizing immunoglobulin G3 antibodies is associated with chikungunya virus clearance and long-term clinical protection. *J Infect Dis* 205: 1147-1154.
159. Scharf O, Golding H, King LR, Eller N, Frazier D, et al. (2001) Immunoglobulin G3 from polyclonal human immunodeficiency virus (HIV) immune globulin is more potent than other subclasses in neutralizing HIV type 1. *J Virol* 75: 6558-6565.
160. Ljunggren K, Broliden PA, Morfeldt-Manson L, Jondal M, Wahren B (1988) IgG subclass response to HIV in relation to antibody-dependent cellular cytotoxicity at different clinical stages. *Clin Exp Immunol* 73: 343-347.
161. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, et al. (2005) Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J Infect Dis* 191: 654-665.
162. Gilbert PB, Peterson ML, Follmann D, Hudgens MG, Francis DP, et al. (2005) Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. *J Infect Dis* 191: 666-677.
163. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, et al. (2006) Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *J Infect Dis* 194: 1661-1671.
164. Chung AW, Ghebremichael M, Robinson H, Brown E, Choi I, et al. (2014) Polyfunctional Fc-effector profiles mediated by IgG subclass selection distinguish RV144 and VAX003 vaccines. *Sci Transl Med* 6: 228ra238.
165. Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, et al. (2008) Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet* 372: 1881-1893.
166. McElrath MJ, De Rosa SC, Moodie Z, Dubey S, Kierstead L, et al. (2008) HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. *Lancet* 372: 1894-1905.
167. Gray GE, Allen M, Moodie Z, Churchyard G, Bekker LG, et al. (2011) Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. *Lancet Infect Dis* 11: 507-515.
168. Hammer SM, Sobieszczyk ME, Janes H, Karuna ST, Mulligan MJ, et al. (2013) Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. *N Engl J Med* 369: 2083-2092.

169. Janes HE, Cohen KW, Frahm N, De Rosa SC, Sanchez B, et al. (2017) Higher T cell responses induced by DNA/rAd5 HIV-1 preventive vaccine are associated with lower HIV-1 infection risk in an efficacy trial. *J Infect Dis*.
170. Letvin NL, Rao SS, Montefiori DC, Seaman MS, Sun Y, et al. (2011) Immune and Genetic Correlates of Vaccine Protection Against Mucosal Infection by SIV in Monkeys. *Sci Transl Med* 3: 81ra36.
171. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, et al. (2012) Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med* 366: 1275-1286.
172. Gottardo R, Bailer RT, Korber BT, Gnanakaran S, Phillips J, et al. (2013) Plasma IgG to linear epitopes in the V2 and V3 regions of HIV-1 gp120 correlate with a reduced risk of infection in the RV144 vaccine efficacy trial. *PLoS One* 8: e75665.
173. Yates NL, Liao HX, Fong Y, deCamp A, Vandergrift NA, et al. (2014) Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. *Sci Transl Med* 6: 228ra239.
174. Johnston MI, Fauci AS (2008) An HIV vaccine--challenges and prospects. *N Engl J Med* 359: 888-890.
175. Gaschen B, Taylor J, Yusim K, Foley B, Gao F, et al. (2002) Diversity considerations in HIV-1 vaccine selection. *Science* 296: 2354-2360.
176. Hatziioannou T, Evans DT (2012) Animal models for HIV/AIDS research. *Nat Rev Microbiol* 10: 852-867.
177. Garcia-Tellez T, Huot N, Ploquin MJ, Rasclé P, Jacquelin B, et al. (2016) Non-human primates in HIV research: Achievements, limits and alternatives. *Infect Genet Evol* 46: 324-332.
178. Barouch DH (2008) Challenges in the development of an HIV-1 vaccine. *Nature* 455: 613-619.
179. Newman MJ (2002) Heterologous prime-boost vaccination strategies for HIV-1: augmenting cellular immune responses. *Curr Opin Investig Drugs* 3: 374-378.
180. Lu S (2009) Heterologous prime-boost vaccination. *Curr Opin Immunol* 21: 346-351.
181. Lu S, Wang S, Grimes-Serrano JM (2008) Current progress of DNA vaccine studies in humans. *Expert Rev Vaccines* 7: 175-191.
182. Munseri PJ, Kroidl A, Nilsson C, Joachim A, Geldmacher C, et al. (2015) Priming with a simplified intradermal HIV-1 DNA vaccine regimen followed by boosting with recombinant HIV-1 MVA vaccine is safe and immunogenic: a phase IIa randomized clinical trial. *PLoS One* 10: e0119629.
183. Brave A, Ljungberg K, Boberg A, Rollman E, Isaguliantis M, et al. (2005) Multigene/multisubtype HIV-1 vaccine induces potent cellular and humoral immune responses by needle-free intradermal delivery. *Mol Ther* 12: 1197-1205.
184. Brave A, Ljungberg K, Boberg A, Rollman E, Engstrom G, et al. (2006) Reduced cellular immune responses following immunization with a multi-gene HIV-1 vaccine. *Vaccine* 24: 4524-4526.
185. Earl PL, Cotter C, Moss B, VanCott T, Currier J, et al. (2009) Design and evaluation of multi-gene, multi-clade HIV-1 MVA vaccines. *Vaccine* 27: 5885-5895.



186. Su L, Graf M, Zhang Y, von Briesen H, Xing H, et al. (2000) Characterization of a virtually full-length human immunodeficiency virus type 1 genome of a prevalent intersubtype (C/B') recombinant strain in China. *J Virol* 74: 11367-11376.
187. Rodenburg CM, Li Y, Trask SA, Chen Y, Decker J, et al. (2001) Near full-length clones and reference sequences for subtype C isolates of HIV type 1 from three different continents. *AIDS Res Hum Retroviruses* 17: 161-168.
188. Clegg CH, Roque R, Perrone LA, Rininger JA, Bowen R, et al. (2014) GLA-AF, an emulsion-free vaccine adjuvant for pandemic influenza. *PLoS One* 9: e88979.
189. Pollara J, Hart L, Brewer F, Pickeral J, Packard BZ, et al. (2011) High-throughput quantitative analysis of HIV-1 and SIV-specific ADCC-mediating antibody responses. *Cytometry A* 79: 603-612.
190. Polonis VR, Brown BK, Rosa Borges A, Zolla-Pazner S, Dimitrov DS, et al. (2008) Recent advances in the characterization of HIV-1 neutralization assays for standardized evaluation of the antibody response to infection and vaccination. *Virology* 375: 315-320.
191. Choudhry V, Zhang MY, Sidorov IA, Louis JM, Harris I, et al. (2007) Cross-reactive HIV-1 neutralizing monoclonal antibodies selected by screening of an immune human phage library against an envelope glycoprotein (gp140) isolated from a patient (R2) with broadly HIV-1 neutralizing antibodies. *Virology* 363: 79-90.
192. Brown BK, Wieczorek L, Kijak G, Lombardi K, Currier J, et al. (2012) The role of natural killer (NK) cells and NK cell receptor polymorphisms in the assessment of HIV-1 neutralization. *PLoS One* 7: e29454.
193. Forthal DN, Landucci G, Phan TB, Becerra J (2005) Interactions between natural killer cells and antibody Fc result in enhanced antibody neutralization of human immunodeficiency virus type 1. *J Virol* 79: 2042-2049.
194. Goepfert PA, Elizaga ML, Seaton K, Tomaras GD, Montefiori DC, et al. (2014) Specificity and 6-month durability of immune responses induced by DNA and recombinant modified vaccinia Ankara vaccines expressing HIV-1 virus-like particles. *J Infect Dis* 210: 99-110.
195. Currier JR, Ngauy V, de Souza MS, Ratto-Kim S, Cox JH, et al. (2010) Phase I safety and immunogenicity evaluation of MVA-CMDR, a multigenic, recombinant modified vaccinia Ankara-HIV-1 vaccine candidate. *PLoS One* 5: e13983.
196. Arthos J, Cicala C, Martinelli E, Macleod K, Van Ryk D, et al. (2008) HIV-1 envelope protein binds to and signals through integrin  $\alpha 4\beta 7$ , the gut mucosal homing receptor for peripheral T cells. *Nat Immunol* 9: 301-309.
197. Peachman KK, Karasavvas N, Chenine AL, McLinden R, Rerks-Ngarm S, et al. (2015) Identification of New Regions in HIV-1 gp120 Variable 2 and 3 Loops that Bind to  $\alpha 4\beta 7$  Integrin Receptor. *PLoS One* 10: e0143895.
198. Nakamura GR, Fonseca DP, O'Rourke SM, Vollrath AL, Berman PW (2012) Monoclonal antibodies to the V2 domain of MN-rgp120: fine mapping of epitopes and inhibition of  $\alpha 4\beta 7$  binding. *PLoS One* 7: e39045.
199. Nilsson C, Godoy-Ramirez K, Hejdeman B, Brave A, Gudmundsdotter L, et al. (2014) Broad and potent cellular and humoral immune responses after a second late HIV-modified vaccinia virus ankara vaccination in HIV-DNA-primed and HIV-modified

vaccinia virus Ankara-boosted Swedish vaccinees. *AIDS Res Hum Retroviruses* 30: 299-311.

200. Sallusto F, Lanzavecchia A, Araki K, Ahmed R (2010) From vaccines to memory and back. *Immunity* 33: 451-463.